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VOLUME 86 NUMBER 6

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

June, 1950

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PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

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This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

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THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

THE USE OF 3,-METHYL, 5, PHENYLHYDANTOIN (AC 114)* IN THE TREATMENT OF EPILEPSY (A PRELIMINARY REPORT)

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Received for publication November 30, 1949

AC 114 (3-Methyl-5-phenylhydantoin) was synthesized by Spielman, and its anticonvulsant properties investigated by Everett (1). Pharmacological studies by Everett and Richards (2) showed that the drug is considerably less toxic than Mesantoin or Dilantin. With doses of 300 mg/kg AC 114 causes slight ataxia in mice and rabbits and affords protection against metrazol convulsions. It also eliminates the tonic phases of supramaximal electro-shock convulsions. In cats the fatal dose is approximately 1 gm/kg. The animals are comatose for 48 hours or longer before dying. A group of dogs have now received daily doses of 50 and 100 mg/kg per day for a period of over six months and are in good health. Thus far, blood counts, bone marrow and liver function tests have all remained within normal limits.

On the basis of these studies (2) indicating a low toxicity and excellent anticonvulsant properties, AC 114 was submitted to us for the initial clinical trial.

MATERIAL FOR STUDY

This study deals with a group of 42 patients of the Epilepsy Clinic of the Johns Hopkins Hospital who were treated with AC 114. Twenty-seven of these patients had been receiving one or more of the usual forms of therapy such as phenobarbital, Dilantin, Mesantoin, Tridione, Benzedrine or bromides for at least one year before AC 114 was started. In each instance the previous medication had proven to be ineffective. Each of these patients was treated with AC 114 plus their previous ineffective medication. The remaining 15 patients had been observed in the clinic for at least six months but did not receive regular anti-

* AC 114 was supplied through the courtesy of Drs. G. M. Everett and R. K. Richards, Abbott Laboratories, North Chicago, Illinois.

convulsant medication. Each of these patients was treated with AC 114 alone.

Thirty-five of these patients suffered from idiopathic epilepsy and seven had organic (secondary) epilepsy. Seven patients had petit mal spells; 23 patients had grand mal seizures; five patients had psychomotor attacks and seven patients had a combination of grand mal, petit mal, and psychomotor seizures.

Thirty of the patients ranged in age from $2\frac{1}{2}$ years to 14 years, and 12 from 14 to 32 years.

DURATION OF TREATMENT

All of the patients were observed for periods ranging from three months to one year. In one patient the medication was discontinued after 10 days trial because of a side reaction. With twelve patients the drug was stopped after three months because it proved to be ineffective. The remaining 29 patients received the drug for a period of three months to one year.

DOSAGE

AC 114 was supplied in a 0.2 gram tablet. The daily dosage of the drug administered to these patients varied from 0.3 gram to 1.0 gram. Generally, with the younger children (under eight years of age) the initial dose of 0.1 gram three times daily was increased until satisfactory therapeutic results were obtained. The daily dose in the younger patients did not exceed 0.6 gram daily. With the older group, including children over eight years of age and the adults, the daily dose varied from 0.6 gram to 1.0 gram. In all instances the medication was administered in three or four doses over the course of the day.

RESULTS

All of the patients followed in this study had suffered with frequent seizures prior to the institution of AC 114 therapy. In each case the number of seizures during the course of AC 114 therapy was compared with a similar observation period just prior to the starting of the AC 114.

In evaluating the response to therapy, the following classification was observed:

1. Controlled: No recurrence of seizures during the entire observation period.

2. Markedly Improved: Number of seizures reduced to less than one-fourth of the previous frequency.

3. Improved: Number of seizures reduced to between one-half and one-fourth of the previous frequency.

4. Failure: Number of seizures only very slightly reduced, or the status remained the same, or the seizures became more frequent.

TABLE I

Results of AC 114 Treatment With Respect to Type of Epilepsy and Type of Seizure

TYPE OF SEIZURE	NUMBER OF PATIENTS	CONTROLLED	MARKED IMPROVEMENT	IMPROVED	FAILURE
Idiopathic					
Petit Mal	6	0	0	0	6
Grand Mal	18	10	4	1	3
Psychomotor	5	0	0	0	5
Mixed	6	1	3	1	1
Organic					
Petit Mal	1	0	0	0	1
Grand Mal	5	3	2	0	0
Psychomotor	0	0	0	0	0
Mixed	1	0	0	0	1
Total	42	14	9	2	17

AC 114 was effective only in controlling grand mal convulsions. As will be seen in Table I, five of the 7 patients with mixed seizures were benefited. All seven of the patients with mixed type of seizures had grand mal convulsions plus either petit mal or psychomotor seizures. In five of these patients the grand mal seizures were controlled. The petit mal or psychomotor spells were not favorably affected in any case.

Of the 42 patients treated, 14 were controlled; 9 were markedly improved; 2 were improved and with 17 the treatment was considered a failure. Of the 25 patients benefited, 10 received AC 114 alone and 15 received the drug in combination with their previous ineffective medication.

Of the 17 patients in whom the seizures were not controlled, five

were treated with AC 114 alone and seven were treated with AC 114 in combination with their previous ineffective medication.

UNTOWARD REACTIONS

Each patient was seen at two week intervals during the first six months of therapy, and thereafter was seen at monthly intervals. White blood cell counts and hemoglobin determinations were done at each visit, and the urine was examined qualitatively for protein, sugar and microscopic elements.

Of the 42 patients treated, only two presented side reactions. Both of these patients developed a skin rash about 10 days after the medication was started. In one instance, the rash was measles-like with an associated pyrexia. The second patient presented a fine generalized erythematous papular eruption without associated fever. With the first case, the medication was permanently discontinued because of the unwillingness of the patient to take the drug again. With the second patient, AC 114 was discontinued when the rash appeared and given again after one week, by which time the rash had disappeared. The rash did not reappear with the second trial.

No abnormal hematological or urinary changes were found. There was no evidence of hyperplasia of the gums; no ataxia nor diplopia was encountered with the doses of AC 114 used in this study. Everett and Richards (2) found AC 114 to have a sedative effect in animals when given in high doses. With the doses employed in our clinical studies, none of our patients complained of lethargy or somnolence.

COMMENT

The results of this study indicate that AC 114 has anticonvulsant properties. In addition to its apparent anticonvulsant value it appears to possess low toxicity and little sedative effect.

It is interesting to note the lack of side reactions and central effects produced by this drug as compared with other chemically related hydantoinates as Dilantin and Mesantoin.

With the doses of AC 114 employed in the treatment of this small group of patients, no evidence of ataxia, diplopia or drowsiness was demonstrated, whereas such reactions are not uncommon with the use of Dilantin or Mesantoin.

AC 114 was found to be effective in a number of instances where other drugs had not given adequate control. It was also used in conjunction with other drugs and no evidence of incompatibility was encountered.

Further study, however, in a large group of cases over an extended period of time will be necessary to substantiate the value and safety of this new drug in the treatment of grand mal epilepsy.

SUMMARY

1. Forty-two patients with epilepsy, 27 of whom were not controlled by other drugs, were treated with AC 114. Twenty-five or 60 per cent were either completely controlled or improved.

2. Only grand mal convulsions were favorably affected.

3. No serious side reactions were encountered.

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AN UNUSUAL CASE OF BACTERIAL ENDOCARDITIS

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Received for publication January 20, 1950

The occurrence of acute osteomyelitis due to the microaerophilic alpha streptococcus is well recognized (1). Its relationship to antecedent infection in the oral cavity or in the upper respiratory tract has been established (2). In recent years reports of large series of cases of bacterial endocarditis due to the microaerophilic alpha streptococcus have appeared, but no mention has been made of the occurrence of acute osteomyelitis as a complicating feature. The present communication deals with a case exhibiting such a relationship.

A. S., JHH #427729, a 59 year old white maintenance worker entered the hospital complaining of excruciating pain in the lower back, radiating in girdle-like fashion into the groins and accentuated by motion. The pain had begun insidiously about one month before admission, increasing in severity and ultimately forcing the patient to move with extreme caution, a "poker spine" being maintained. He had always enjoyed excellent health although told by a doctor some twenty years previously when being examined for insurance that he had a "leaky" heart. He had never had ill effects from this.

About one month before admission the patient developed a mild cold and sore throat which persisted. He noticed one morning on arising a nagging pain in the back when he moved about. This progressed in severity becoming knifelike in character. There was anorexia, loss of twenty pounds in weight, and excessive fatigability. At no time did he feel feverish or chilly. One week before admission he noticed swelling of his ankles. There was orthopnea of increasing degree. Two nights before admission he was awakened from a sound sleep by coughing and a smothering sensation being forced to sit by an open window to get his breath.

Physical examination on admission revealed the appearance of chronic illness. He had a low grade fever. There was pallor and evident weight loss. The skin was clear except for some cherry angiomata over the chest. The posture was striking, the patient holding his back

rigid, guarding every movement. There was marked spasm of the paravertebral muscles. No localized tenderness over the vertebrae was noted. Dental caries was present. The chest was emphysematous. A loud leathery friction rub was heard in the right axilla and numerous moist rales were heard at both bases. The heart was enlarged and had a "mitral" configuration. A systolic thrill was present at the apex. The sounds were of good quality. P2 was accentuated. There was a loud harsh systolic apical murmur and a short, softer low pitched

CHART I

Hemogram:

RBC	4.4 million	WBC	13,600
HGB	13.0 gms.	Juv	3%
		Polys	74%
HT	40.0	Eos	2%
		Lymph	17%
SR	50/36	Monos	4%

Urine and Renal function tests:
Clear yellow, SG 1020, 1+ albumin
Microscopic—1–2 RBC/HPF, 0 WBC, 0 casts
PSP excretion 65% in two hours
IVPs normal

Tuberculin Test:
2nd strength PPD 1+ 24 hours

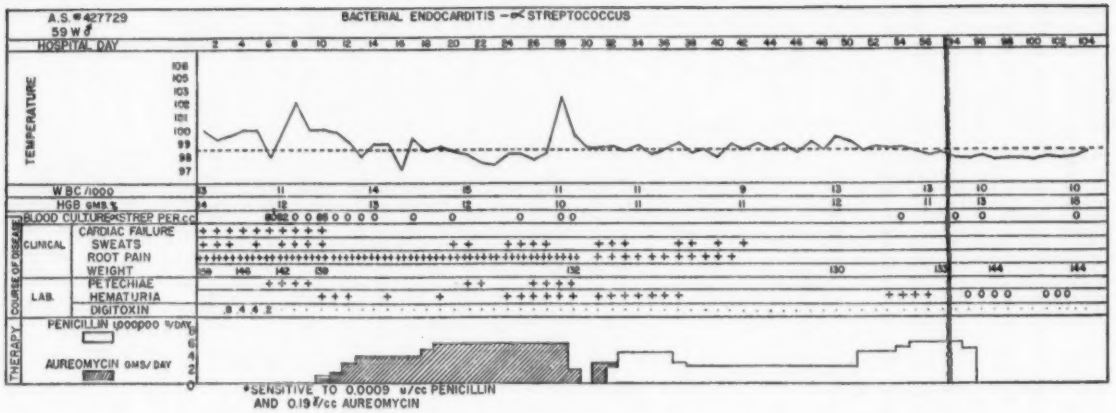
X-ray examinations:
Ba enema and GI series—normal
Long bones, pelvis—normal

Lumbar Puncture:
Crystal clear fluid under normal pressure 1–2 mononuclear cells per mm^3. Sterile. No tubercle bacilli seen on smear or demonstrated by Guinea pig innoculation.

diastolic murmur transmitted into the left axilla. Numerous extrasystoles were present. The liver edge was felt three fingerbreadths below the costal margin. The prostate was soft, boggy, and about two times normal size. The neurological examination was normal except for conduction deafness on the right. The initial laboratory findings are recorded in Chart I.

The patient obviously had rheumatic heart disease with mitral valve involvement and mild cardiac failure, but complicating this was a fever of unknown cause and a destructive lesion of the 8th thoracic vertebra and intervertebral disc as demonstrated by radiological examination. Several possibilities were considered to explain the findings.

CHART II


A.S. # 427729
59 W ♂
BACTERIAL ENDOCARDITIS — α STREPTOCOCCUS
HOSPITAL DAY
TEMPERATURE
WBC/1000
HGB GMS.%
BLOOD CULTURE α STREP PER CC
COURSE OF DISEASE
CLINICAL
CARDIAC FAILURE
SWEATS
ROOT PAIN
WEIGHT
LAB.
PETECHIAE
HEMATURIA
DIGITOXIN
THERAPY
PENICILLIN 1,000,000 U/DAY
AUREOMYCIN GMS/DAY
*SENSITIVE TO 0.0009 u/cc PENICILLIN
AND 0.19 γ/cc AUREOMYCIN

Most observers felt the patient had metastatic carcinoma and rheumatic heart disease. The low grade fever was attributed to the carcinoma. A careful search was made for a possible source of carcinoma, but none was found. On the seventh hospital day several petechiae were

FIG. 1. X-ray of the thoracic spine (1-22-49) showing bone destruction of the anterior inferior lip of T-8 and almost complete loss of the intervertebral disc space between T-8 and T-9.

noted over the trunk and a Roth spot was seen in the left fundus. At the same time the first two blood cultures drawn the day of admission were found to be positive, the organism being identified as a microaerophilic alpha streptococcus. It then appeared that the patient had subacute bacterial endocarditis and that the osteolytic lesion in the 8th thoracic vertebra was an osteomyelitis secondary to an infected embolus.

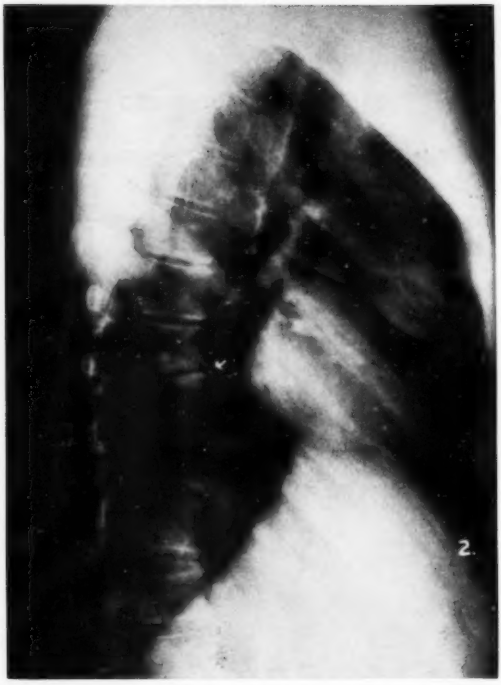


FIG. 2. Same as above (3-31-49) showing beginning repair of the lesion in T-8 and early fusion of T-8 and T-9.

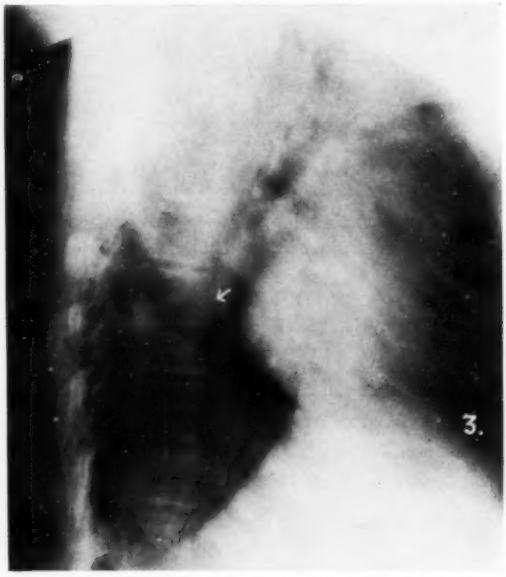


FIG. 3. Same as above (12-5-49) showing fairly complete repair of the lesion in T-8 and fusion of T-8 and T-9.

The patient's hospital course is best followed by Chart II. The cardiac failure responded promptly to the administration of digitalis, a salt poor diet, and rest in bed. The patient was first placed on aureomycin, but failed to show a satisfactory clinical response. Penicillin therapy was then substituted and given during a ten week period, during which time the patient progressively improved. Fever, night-sweats, and back pain disappeared, appetite improved, and body weight increased. The leucocytosis disappeared and the hemoglobin concentration rose. The lesion in the 8th thoracic vertebra showed regression and evidence of healing (figures 1 through 3). The patient was discharged from the hospital after 15 weeks. Since that time he has been followed in the out-patient department and has improved steadily. Subsequent x-ray examination of the spine showed complete healing of the bony lesion.

SUMMARY

1. A case of subacute bacterial endocarditis due to a microaerophilic alpha streptococcus is presented with a hitherto undescribed complication, an acute osteomyelitis of a thoracic vertebra.

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VENOUS VELOCITY IN THE LEG MEASURED WITH RADIOACTIVE SODIUM*

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Received for publication February 4, 1950

INTRODUCTION

The serious problem of venous thrombosis and its graver complication, pulmonary embolism, is today a trenchant challenge to modern medicine. It is an issue of great importance which should be pursued by the concerted inquiry of physicians and surgeons alike. Belt,[4] reporting from the Toronto General Hospital, and White[8] et al, reporting from the Massachusetts General Hospital, both concluded that pulmonary embolism, usually regarded as a postoperative complication, is more often associated with medical than surgical cases. Massive pulmonary embolism (and its precursor venous thrombosis) kills more people than cancer of the stomach. Careful anatomic study has verified the fact that the deep veins of the leg are the most frequent site of venous thrombosis, as well as the most prolific source of both fatal and non-fatal pulmonary emboli. The *in vivo* clotting of blood in the veins of the lower extremities is an abnormal and possibly preventable potential catastrophe. Conclusive evidence indicates that the cure of most pulmonary emboli consists in the prevention of venous thrombosis. Yet it is often difficult or impossible, even with the utmost clinical vigilance, to detect the early signs of silent phlebothrombosis by methods of diagnosis currently in use.

The essence of enlightenment concerning venous thrombosis lies, firstly, in a thorough basic knowledge of the many factors concerned in the kinetics of venous flow of blood from the lower extremities; secondly, in the application of factual biochemical knowledge to the clinical problem of blood clotting; and lastly, in the confession of our ignorance concerning certain of the basic facts of venous hemo-dynamics. The tragic sequelae of thrombosis and embolism are prevent-

*The radiosodium 24 used in this work was obtained on allocation from the United States Atomic Energy Commission.

able and should never be accepted with the same inevitable resignation with which our forefathers accepted "laudable pus".

Venous blood flow from the lower extremities is currently believed to be dependent upon the following factors:

I Segmental linear venous velocity as a component of circulation time.
II Volume flow (dependent upon the sectional area of the venous vascular bed).
III Cardiac output.
IV Anatomic or mechanical factors intrinsically related to veins and vessel wells.
V Muscular "milking-action" on the veins of the lower extremities.
VI Intermittent negative pressure normally present within the abdomen and thorax.
VII Gravity.
VIII Total blood volume.

With the advent of man-made atoms a new method became available for the study of linear venous velocity. This investigation was undertaken to determine whether thrombosis of the deep veins of the leg produces an alteration in the rate of venous return from the lower extremity. This alteration might well be reflected by the rate of transport to the groin of a small amount of radioactivity deposited into the superficial greater saphenous vein at the ankle.

Although it would seem logical to measure this velocity in the deep veins it was thought that the necessity of an incision in order to reach this venous system would limit the clinical application of this procedure. Further, if a significant change could be found in the venous flow in the superficial system in the presence of a deep thrombosis, this simple technique would afford considerable aid in the diagnosis of phlebothrombosis. To obtain a convenient clinical figure this rate of transport was expressed as the linear venous velocity in the greater saphenous vein. With full realization that the tortuosity of the vessel, its liberal anastomoses, the milking action of muscles, and other factors known to exert a similar influence, this value is more properly an index of the rate of venous return than a true linear velocity. Nevertheless, in the presence of occlusion of the deep veins of the leg, al-

teration in the rate of return might be expected to occur in the greater saphenous vein. This preliminary report deals with a method for the measurement of this value and observations under normal conditions. To H. Payling Wright (24, 25) and her colleagues at the University College Hospital in London belongs the credit for having studied and published the original direct measurements of venous circulation time in the lower extremity by means of radiosodium 24. Our investigations begun at about the same time have proceeded at a slower rate due to the unusual difficulty we encountered in enlisting normal subjects as volunteers for tracer studies. The prophecy of this method perhaps rightly belongs to Blumgart and Weiss (5, 6, 7) who in 1927 first measured circulation time with intravenously injected radium emanations.

As noted by Moore (14), Aebersold (1), Warren (22), Lawrence (11) and many others the period 1939–1945 constituted the fledgling days of cyclotron produced isotopes—a small amount of radioactivity created at a great cost. A few intrepid investigators spurred on by callow curiosity began to explore the field of nucleonics in medicine. Recent atomic energy developments have now make pile-produced isotopes—the by-products of war-borne A-bombs—available as extremely useful tools in medical and scientific research on a wide scale and at a low cost.

METHOD

By definition the linear venous velocity is the rate of venous blood flow along a given segment of vein. In very small transparent vessels, as in the mesentery, or toe-web of a frog and the cornea of the eye in man, the blood velocity can be measured by timing the progress of a red cell beneath the microscope. In larger vessels the velocity could heretofore only be calculated if the quantity of blood which passed a given point in a unit of time and the cross sectional area of the vessel were precisely known. Such knowledge was imperfect at best. Merely severing a vein of known size and measuring the amount of blood which escapes over a given period of time results in a completely inaccurate conclusion for it changes two of the most important factors in venous blood flow, namely the volume flow and the venous pressure relationships.

Several instruments have been devised for measuring the volume of blood flow in animals. The stromuhr of Ludwig is one of the oldest and best known physiologic instruments, and the thermostromuhr of Rein is a more recent and perhaps more precise method of measurement. However, until the advent of radioisotopes and the

development of tracer techniques there had been no reliable or expedient method for determining the segmental linear velocity of blood under physiologic conditions.

Tests which have hitherto been used to determine circulation time (velocity of blood flow including venous, pulmonary and arterial vascular components) have for the most part been subjective tests requiring the cooperation of the patient and being limited by the error of individual reaction. Radioactive sodium, however, can be safely administered by intravenous injection in tracer amounts and its journey through the veins accurately charted with objective precision.

Radioactive sodium 24 has a half life of 14.8 hours. Potent gamma and less penetrating beta radiations are given off which have been found useful in a number of biological and clinical studies. Sodium is fairly uniformly distributed throughout extracellular body fluids. When radioactive sodium is administered intravenously it emits a penetrating radiation which can be traced by means of a Geiger counter. After complete disintegration of radiosodium within a period of four days a normal inactive atom remains which is perfectly harmless and devoid of any delayed or permanent injurious after-effects.

When working with radioisotopes, however, it is essential that the most effective health-safety practices be strictly adhered to. Toward this end the general rules and monitoring devices recommended by the Atomic Energy Commission's "Standard Safety Requirements" (20) were used in our work as a guide against the hazards of radiomaterials.

Radiosodium, Na 24, as supplied by the A.E.C., is chemically pure sodium carbonate. One irradiation unit contains 0.3 grams of sodium carbonate with an estimated radioactivity of 20 millicuries. For the purpose of our studies it was found expedient to receive only one-half an irradiation unit.

By careful telemanual technique the dry radiosodium carbonate was transferred into a small sterile beaker. Hydrochloric acid was added to bring the solution to pH 7.0 and the volume adjusted to 4 cc. The resulting neutral but somewhat hypertonic solution was used in amounts of 0.1 cc. to 0.2 cc. in order to avoid any appreciable effect upon venous blood flow.

As pointed out by Quimby (17) the amount of radiosodium to be used in human studies must be small enough so as not to constitute a danger to the subject or to distort the experimental findings. On the basis of Marinelli's (12) studies 1 microcurie of radiosodium per kilogram of body weight delivers a total body radiation of about 0.11 r over an effective period of about two days, if both beta and gamma rays are considered. The accepted tolerance dose for continuous total body exposure is 0.1 r daily. Thus, it is safe to use tracer doses of radiosodium amounting to 2 or 3 microcuries per kilogram of body weight. In the present study the tracer amount of radiosodium used varied between 1 and 2 microcuries per kilogram of body weight, never exceeding a total dose of 75 microcuries and never being repeated at short intervals. Bacteriologic studies indicated that radiosodium as received in its sealed aluminum container was sterile. Berkefeld filtration, however, was practiced.

The subjects of this study were for the most part young healthy adult volunteers (medical students, nurses, technicians and patients in well-being) who were allowed to rest at room temperature for 30 minutes (basal condition) before performing the test. The subject's entire leg was exposed and the heel was propped up on a sand-bag so that the level of the site of injection was approximately horizontal with the level of the fossa ovalis.

The site of injection was chosen as the distal termination of the greater saphenous vein at a point adjacent to the medial malleolus. Either leg was employed

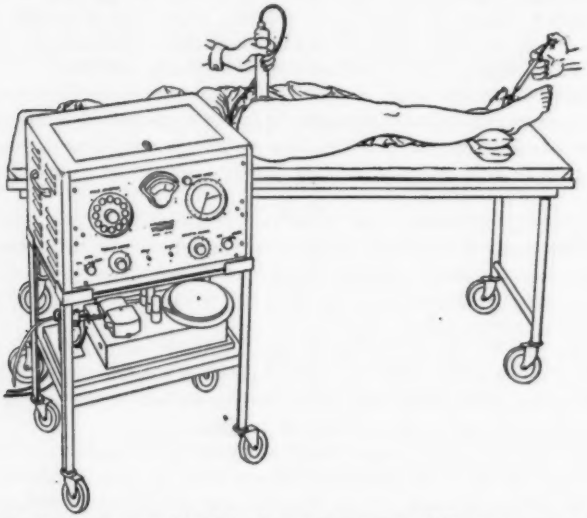

FIG. 1. Apparatus employed in the measurement of linear venous velocity in the lower extremity. Mounted beneath the Geiger-Muller counter is the electronic recording and timing device described in the text. Shielded G-M tube now held by stand without touching leg.

and the linear distance between the site of injection and the fossa ovalis was carefully measured. With the Geiger-Muller tube (a lead-shielded G-M tube was used in later work) held in place over the fossa ovalis without pressure on the underlying tissues, 0.1–0.2 cc. of radiosodium was injected into the greater saphenous vein at the ankle. A tuberculin syringe and a 25 gauge hypodermic needle were used. The precise time of injection was noted by an assistant on a recording tape which, by means of an electronic circuit connected to the Geiger counter, visibly recorded the counts. The scaler and recorder were so arranged that every thirty-two counts were indicated by a transverse line on the tape. One second intervals were simultaneously recorded. (Fig. 1)

Prior to the arrival of radiosodium at the inguinal region the Geiger counter picked up a rather constant background count which consisted of cosmic radiation and stray radioactive scattering. This rate was of the order of one count every one or two seconds and in no way interfered with the sudden rapid rise of counts which signaled the arrival of radiosodium at the fossa ovalis. The increase in the counting rate when the radiosodium flowed under the Geiger-Muller tube averaged about several hundred counts per second. Thus, the time interval in seconds between the injection at the ankle and the arrival at the inguinal region denoted the rate of venous return flow in the lower extremity. (Fig. 2)

In order to determine the precise time of arrival of radioactivity under the tube, the data were plotted on a semilogarythmic scale as indicated in Figure 3. The point at which there was a sharp change in the slope of the curve of recorded counts was considered the time of arrival of the radioactivity at the groin.

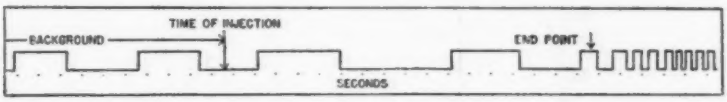

FIG. 2. Reproduction of the recording on paper tape of a typical determination of linear venous velocity. C. A.V., a 22 year old medical student, received 0.1 cc. containing 50 microcuries of radioactive sodium 24 at the ankle. The time between the injection and the end point is 17.1 seconds.

RESULTS

The linear venous velocities were determined in 40 subjects, of which 20 were young, healthy adult volunteers and 20 were hospital patients admitted for various diseases, but at the time of study were in a state of well-being. The mean venous velocity of the 20 normals was not significantly different from the 20 hospitalized patients when subjected to statistical analysis, and for this reason it is believed that these 40 determinations represent a valid normal series. The mean velocity for the entire group was 3.8 $\pm$ 0.37 cm. per second. As demonstrated in the accompanying Figure 4, these results describe a skew distribution with a sharp rise from 1.3 to 2.0 cm. per second but with considerable spread in the more rapid velocity group.

Examination of the data fails to reveal any difference in the velocities between the two sexes or any variation dependent upon age between twenty and seventy years. No effort was made to determine the effect of vasodilators or temperature variations on the rate of venous flow.

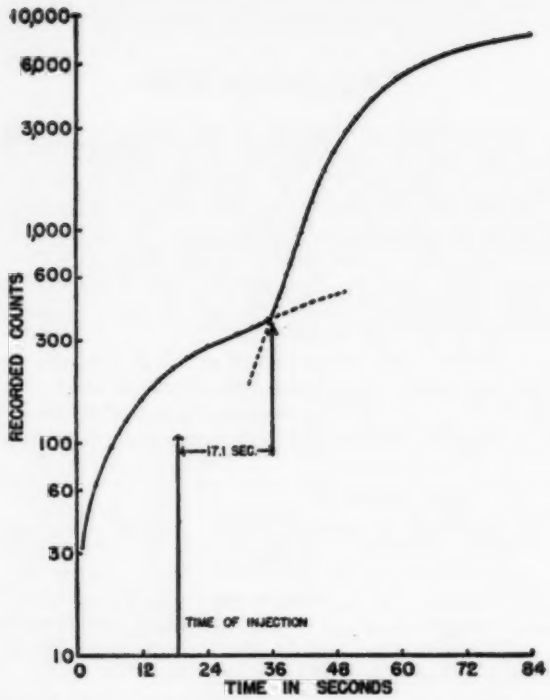

FIG. 3. Graph of accumulated counts on subject C.A.V. The marked change in the slope of the curve reveals the time of arrival of the radiosodium under the Geiger-Muller tube at the fossa ovalis. The time between the injection and the end point is 17.1 seconds.

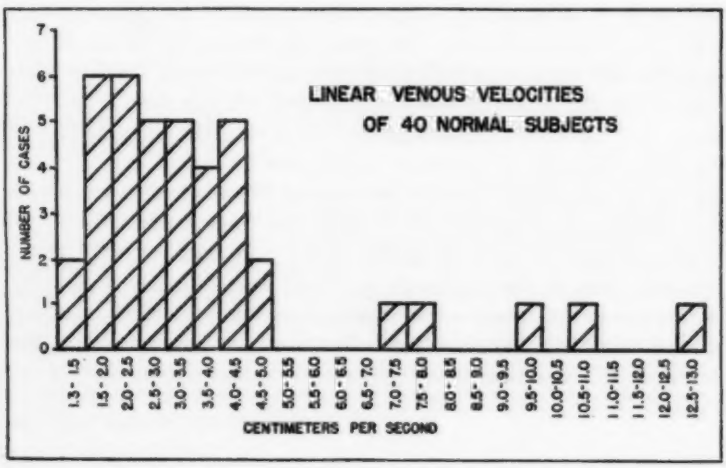

FIG 4

In 18 cases a peculiar localization of radioactivity was found over the course of the injected vein on the medial aspect of the calf of the leg about midway between the ankle and the knee. In the entire 40 cases there was a small residue of radioactivity for a short period of time at the site of injection and a gradual decrease in the intensity of this activity was readily noted as the G-M tube was moved progressively up the leg. However, in these 18 cases there was an abrupt increase in activity as the counting tube reached an area about 3 centimeters in diameter at the mid-calf. The persistence of radioactivity at this site was unaffected by leg or body posture or twenty minutes of walking in the 6 cases where this was studied. It was still present but decreased in amount three and a half hours after injection in one case, and twenty-four hours later in another. In one case not included in this series an opportunity occurred to dissect such a leg which had repeatedly revealed puddling of a few hours duration. This leg was amputated because of arteriosclerotic gangrene of the foot shortly after the injection of radioactive sodium. Our search for factors that might possibly play a role in this unusual phenomenon revealed a venous tributary plexus entering the greater saphenous vein in the calf of the leg at a most unusual angle. At the very site of puddling the tributary vein entered the greater saphenous vein at an obtuse angle which it is believed could produce eddy currents at this site. However, it must be noted that in most cases the puddling persisted for a period far too long to be satisfactorily explained by these eddy currents alone. The cause of this puddling phenomenon is obscure, but further studies are in progress.

DISCUSSION

As suggested recently by Arthur Allen,[2] it seems quite apparent that the entire problem of thrombosis and embolism should be considered in two distinct categories, namely, (1) prevention and (2) treatment. "That treatment of thrombosis and non-fatal emboli is reasonably effective by the intelligent use of femoral vein interruption, procaine sympathetic blocks, and anticoagulants is well established." However, the primary problem—how best to prevent thrombosis—remains to be solved. If permanent and enduring benefit is to be expected, this serious challenge must be successfully met.

The importance of general non-specific measures available in the prevention of venous thrombosis have been commented upon by many authors. The use of early postoperative active and passive leg motion, the careful regulation of the nutritional state, adjustment of water, salt and protein balance, and a deep breathing routine have all been considered helpful in reducing the incidence of thrombosis and embolism. Of added benefit in prophylaxis can also be mentioned such generally accepted features as, elevation of the foot of the bed when feasible, the use of elastic leg bandages, and early postoperative ambulation. If, however, so-called "bland" thrombosis has already occurred Homans[9] has advocated interrupting the deep veins of the leg as a likely means of preventing recurring pulmonary embolism. The use of anticoagulants after phlebothrombosis has already manifested itself has also been successful in diminishing postphlebitic sequelae notably, pulmonary embolism. To Ochsner and DeBakey[15] and their interest and enthusiasm we owe the application of the valuable method of treatment by repeated procaine blocks of the lumbar sympathetics.

Virchow,[21] Aschoff[3] and Welch[23] were among the first to consider the retardation of the circulation as a most important cause of thrombosis. Almost a century ago Virchow stressed the fact that an enfeebled circulation in addition to eddy currents produced by venous valves, tributary veins and alterations in the calibre of the vessels probably plays a part in the origin and propagation of intravenous clotting. Yet expected pathologic changes in the vessel wall beneath the site of thrombus formation are conspicuous by their absence. In a thorough post-mortem study of fatal pulmonary embolism Patey[16] concludes, "The veins in these subjects are exasperatingly normal."

Although most medical opinion concedes the importance of venous stasis as a factor of primary concern in contributing to the development of thrombosis and embolism, yet the fundamental facts of the mechanical aspects of venous stagnation have received only limited study. The objective measurement of circulation time has interested many medical investigators for a number of years. Most methods require the cooperation of the subject, and while lacking precision, have contributed to our understanding of total blood flow—both arterial, pulmonary and venous. The present study, however, reports a precise, direct and totally objective method of measuring linear venous

velocity in a particular segment of the vascular system. Subjective errors by either the patient or the observer are reduced to a minimum by the use of an electronic recording device calibrated in counts per second and connected to a G-M counter.

The mean linear venous velocity in 40 normal subjects was found to be 3.8 ± 0.37 centimeters per second when measured from ankle to groin. Although 88 per cent of these blood flow determinations were between 1.3 and 5.0 centimenters per second, yet the extremes of venous velocity ranged from 1.3–13.0 centimeters per second. This normal variation of linear venous velocity under standard conditions of rest and temperature is greater than might be anticipated. Whether or not this relatively wide variation among normal subjects will impair the value of this test as an aid in the early diagnosis of venous thrombosis remains to be seen. Future studies are contemplated using (1) postoperative cases, (2) medical cases subject to the hazards of prolonged bed rest, and, if possible (3) cases presenting the clinical evidence of venous thrombosis and pulmonary embolism. The observations of Smith and Allen[18] noting that the period of maximal slowing of venous circulation corresponds to the postoperative period in which phlebitis and pulmonary embolism occur most commonly merits critical inquiry. Their results indicating that "82 per cent of individual patients showed significant slowing of blood flow in the foot-to-carotid sinus pathway after operation while the arm-to-carotid circulation time changes very little" is interesting in relation to the predilection of thrombosis for the lower extremities. Verification of these findings using tracer techniques is certainly indicated.

It is, of course, difficult to compare linear venous velocity in the lower extremity as measured by radiosodium 24 with published reports[13, 19] of partial circuit circulation time studies. The indirect methods of Kvale, Smith and Allen[10] report the average "arm to carotid sinus time" as 20.8 seconds; the "foot to carotid sinus time" as 39 seconds. The ankle to groin time in our group of 40 subjects when calculated on the basis of 80 centimeters as the mean distance, averaged 21 seconds which may bear a comparable relation to the blood flow both in arteries and veins in the circuit reported above.

The accuracy of this method more closely parallels the original and significant work of Wright, Osborn and Edmonds.[24, 25] However, in

computing our data to conform with the foot-groin time of Wright et al we find that their measurement of this segmental venous velocity is several seconds faster than ours. This, however, may very well be explained by minor variations in the two techniques. The English investigators used a very small amount of radioactivity in a 1.0 cc. volume injected through a 20 gauge needle as rapidly as possible. In order to avoid interference with normal hemodynamics 0.1–0.2 cc. was injected through a 25 gauge needle in this study. The injection time was almost always less than one second.

It is of interest to note that the values of Wright et al describe a skew distribution curve, but that their variation was in the slower rates of venous velocity, whereas our variation appeared in the more rapid velocities.

The perplexing observation of puddling of the radiosodium 24 in the mid-calf region noted in 45 per cent of normal subjects leaves us benighted. Venous eddy currents, silent thrombi, stagnation of unknown origin, deposition of blood-elements on the venous walls and extravasation have all been conjured up as possible explanations. Reasons for this radioactive reservoir are being studied.

SUMMARY

(1) A method for the determination of segmental linear venous velocity employing radiosodium 24 is presented. In a series of 40 normal subjects this velocity as measured from ankle to groin was found to be 3.8 $\pm$ 0.37 centimeters per second.

(2) In this series 88 per cent of the venous velocity determinations were 1.3–5.0 centimeters per second with extremes of 1.3–13.0 centimeters per second. Age and sex was not observed to influence the rate of flow.

(3) In 45 per cent of these normal subjects radiosodium 24 was found to persist in the region of the mid-calf following injections. This puddling phenomenon was not influenced by posture and exercise and in 1 case was observed twenty-fours after injection.

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THE CLINICAL PICTURE ASSOCIATED WITH INFECTIONS DUE TO CRYPTOCOCCUS NEOFORMANS (*TORULA HISTOLYTICA*)

REPORT OF THREE CASES WITH SOME EXPERIMENTAL STUDIES

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Received for publication February 4, 1950

Although the yeast-like organism now designated as Cryptococcus neoformans (or hominis) and perhaps better known as Torula histolytica is widespread in nature, infections due to it are evidently rarely encountered or, at least, rarely recognized. However, under suitable conditions this organism may invade the human body, producing infections that may either be localized and relatively benign or progressive and leading in the great majority of patients to a fatal outcome. The present report is concerned with three instances of this disease which serve to illustrate this tendency of the cryptococcus to produce a clinical picture falling into one of the two categories mentioned above.

I. GENERAL CONSIDERATIONS

(1) *Mycology:* The cryptococcus is a yeast-like organism which has been found in the juice and surface of peaches, on various plants, in the bodies of many insects, in wasps' nests and in milk, etc. It has also been found on human skin, in the throat and gastro-intestinal tract, and has been recovered from infections in the horse and some other animals. It grows readily at room temperature or at 37° C. as a moist, slimy growth that appears in about 2 days as whitish or cream-colored colonies which become orange or brownish in about one week. The organisms are ovoid to spherical, are yeast-like in appearance and size (though varying greatly in size), have wide gelatinous capsules and produce single buds. The capsules are best demonstrated by examining the pus or the culture with dilute India Ink. The cryptococci may be differentiated from budding yeasts such as blastomyces by their formation of single buds, their wide capsule and their failure to

produce a mold-like growth or mycelia at room temperature. The virulent cryptococci are pathogenic for various laboratory animals—especially mice—and on intraperitoneal inoculation in this species produce a fatal infection in 3 to 4 weeks with gelatinous masses in the mesentery, enlarged lymph nodes in the peritoneal cavity and lesions in the brain and other organs (1, 2, 3). No instance has been recorded of infection in laboratory workers from infected animals or cultures, nor of cross infections from animal to animal or man to man.

(2) *Clinical and Pathological Features:* There have been several articles giving excellent clinical descriptions of the disease cryptococcosis as well as good descriptions of the pathological findings (1–17). From a consideration of these data we can recognize this infection as a chronic or subacute one that may either remain localized for long periods of time, finally clearing up without further spread, or one which slowly invades the tissues of the body, then spreading by the lymphatics and blood stream to become implanted in various tissues and organs, always sooner or later showing an especial affinity for the central nervous system. When meningitis develops the course is more rapid and nearly always fatal. It is also apparent that the cryptococcus grows slowly in man, indicating that man is probably not a suitable or natural host for this parasite which has the properties of a secondary rather than primary pathogen. The portal of entry has not been established, though the consensus of opinion seems to favor the respiratory tract as the most likely route. The *pathological* lesions in man are characterized by their tendency to stimulate little inflammatory response and the lesions have a gelatinous appearance due to abundance of capsular material. In skin lesions the cryptococci may be found without abscess formation and the appearance of involved lymph nodes may simulate that of Hodgkin's disease. In the brain, tumor-like masses of the organisms may be found at times with apparent cyst formation and without much cellular reaction (1, 2, 3). In the meninges the cryptococci produce a true meningitis with or without granulomata and the organisms are usually abundant in the exudate. In the brain they may occur in the gray matter or basal ganglia and frequently form cyst-like areas due to the capsular material. Epithelioid and giant cells at times are numerous in these lesions. In the lungs similar pathologic changes are found, again with little

surrounding cellular reaction, often with epithelioid and giant cells and with large gelatinous collections of the cryptococci. Other organs or tissues which are frequently involved are the skin and subcutaneous tissue, and less frequently the kidneys, liver, spleen, adrenals or pancreas (2). Rawson et al (18) found reference to 8 cases with adrenal involvement and reported one of their own in which there was adrenal insufficiency.

The diagnosis of cryptococcosis is frequently missed during life because of its comparative rarity and because it is often mistaken for other types of infections. When localized lesions are drained the organisms may escape notice in the pus unless looked for specifically, and on the usual culture media their growth may be too slow to be observed by routine diagnostic methods. In the spinal fluid they are often mistaken for lymphocytes unless one notices the single budding forms and the capsules. The X-ray changes in the lungs are non-specific and could be produced by a variety of conditions. The diagnoses, therefore, that are most frequently entertained are tuberculosis of lungs or meninges, lymphocytic chorio-meningitis, syphilitic meningitis, various types of meningo-encephalitis, brain abscess, brain tumor, Hodgkin's disease, blastomycosis, actinomycosis, etc.

(3) *Animal Susceptibility:* Some animals are quite susceptible to infection with virulent cryptococci, notably the mouse, guinea pig and rat, whereas the rabbit is relatively resistant. Kuhn (19) feels that the high normal temperature of rabbits and the higher temperature which they develop as a result of the infection may be an important factor in this animal's resistance, as the cryptococci were found in vitro to be markedly inhibited in growth or killed by a temperature of 105° F. for 3 hours and only a few survived a temperature of 107° F. for one hour. Most of the experimental work on animals has been done in mice; in them after intraperitoneal injection the lesions are found in the peritoneum, and later in the liver, spleen, kidneys, lungs, meninges and occasionally in the brain. In experiments on mice, Wade and Stevenson (7) found that large mononuclear cells were present in most of the lesions and that many of these cells contained torulae. Dogs have also been infected experimentally by intravenous injection, and in them the cryptococci have been found to produce lesions in the brain and kidneys only (2).

(4) *Peculiarities of the Infection:* There are several features of this disease for which an adequate explanation has not been found. One of these has been mentioned before, namely the rarity of the clinical infection in man despite the widespread distribution of the cryptococcus in nature. Allowing for the fact that many cases are not diagnosed clinically or even pathologically because of lack of awareness of the possibility of this type of infection, still the appearance in the literature of only about 120 case reports (17) certainly indicates a low incidence. This means that the majority of the strains at least show only a low pathogenicity for man.

Another finding which has been remarked on is the relative frequency of the occurence of cryptococcosis in association with Hodgkin's disease. One such case was reported by Warvi and Rawson (10) and another by Tinney and Schmidt (11), who state that 8 other similar instances have been recorded. It has also been noted in association with tuberculosis.

Another feature which deserves further study is that having to do with the *immunological* response. Hoff (20) found it difficult to demonstrate hypersensitivity in patients with the infection by tests in skin or serum, and tests in animals resulted in only minor immunological reactions. Conant (1) mentions the fact that antibodies in the form of agglutinins and complement fixing antibodies are usually not found in the human infection even after vaccine therapy. He refers to the work of Benham (21), however, who found that antibodies could be produced in rabbits by injecting them with cells from which the capsular material had been removed. This method was employed by her for the classification of the various types of cryptococci. Cox and Tolhurst (3) obtained irregular results in their studies on the organism's antigenic behaviour, but were able to produce some cross agglutinins and precipitins in rabbits' sera against the strains of cryptococci which they have isolated from infections in man. The studies of Kligman (22) are likewise of especial interest in this regard. He removed the capsular substance from the cells and found that it was a polysaccharide which was inactive in producing precipitins or skin hypersensitivity in rabbits; injections into mice did not confer any immunity toward subsequent inoculation with living cells. He also failed to obtain a serologic response or evidence of skin sensitivity in rabbits, mice

or rats injected either with whole living cells, decapsulated cells or those killed with heat or phenol. Neill et al (23), however, produced antisera in rabbits by injection of cryptococcus cultures and found that these antisera reacted with the purified polysaccharide of the capsular substance in precipitin tests at high titers, caused agglutination of the cells and reacted specifically with the capsules of the organisms. This latter phenomenon as observed microscopically was not a typical "Quellung" of the capsules with visible enlargement, but was a reaction that made the capsular border appear more sharply delineated. In some strains of cryptococci having narrow capsules difficult to see by the usual techniques, capsules could thus be more readily demonstrated.

The question of effective *therapy* of cryptococcus infection presents another problem that is far from settled. Moreover, it is hard to judge the results of therapy in a disease such as this in which the lesions may be either localized or disseminated, and in which the infection may run a chronic course with natural remissions, at times lasting for many months; or in which the course may be progressive from the time the infecting agents first gain access to the tissues of the host. Suffice it to say at this point that no chemotherapeutic or antibiotic agent has as yet been proved to be of real value in the experimental infection or in man. The same holds true for vaccine therapy. The iodides are routinely employed as in other similar infections, but with unsatisfactory results except in an occasional case. E. L. Jones (5) reported the results of therapy in a patient with a destructive lesion of the nasopharynx in whom iodides, arsenic and ultraviolet light were ineffective. In this patient X-ray therapy apparently resulted in arresting or curing the condition. S. H. Jones and Klinck (24) reported a case of meningitis in which iodides, gentian violet (intravenously) and thymol (intramuscularly) were used without benefit. Beck and Voyles (25) obtained negative results in a series of experiments in mice infected with cryptococci; these animals were treated with potassium iodide, with sulfadiazine, and with a combination of the two drugs. The results with the sulfonamides clinically have been variable and on the whole discouraging, though an occasional case has seemed to be benefited. Marshall and Teed (26) treated a patient who had bilateral mastoiditis and meningo-encephalitis due to a cryptococcus;

the course of this patient's disease was apparently arrested following bilateral mastoidectomy, the local application of sulfanilamide in the mastoid wounds and systemic treatment with sulfadiazine. Conant (1) obtained good results with sulfadiazine in a patient with extensive pulmonary lesions and Reilly and Artman (27) felt that the sulfonamides (particularly sulfadiazine) had been of some help in controlling the infection in a chronic case observed by them over a nine year period, though the patient was not considered cured. Keeney et al (28) found that the Cryptococcus neoformans in vitro was inhibited by sulfonamides only at a level of 1000 mgs. per cent or more. Jones and Klinck (24) found that sulfadiazine did not alter the course of the disease in their patient with meningitis—though they only gave it for 4 days. Experiments which they conducted in vitro and in mice also proved to be negative. Flinn et al (14) found no evidence of therapeutic effect in their case in whom sulfathiazole, sulfadiazine, potassium iodide and antimony and potassium tartrate were employed, at various times. In vitro tests with several sulfonamides were likewise negative. Daniel et al (17) found that the growth of cryptococci was inhibited by "soluthiazole" (sodium sulfathiazole) at a strength of 20%, but not at 10%. They also noted high in vitro activity of propamidine and of crystal violet.

In an early report on penicillin, Dawson and co-workers (29) found that it was effective against the Cryptococcus neoformans in vitro. However, subsequent studies in vitro (12,24,28) and in mice (24) gave little if any promise of its therapeutic efficacy. Reilly and Artman (27) found that mice treated with penicillin, sulfadiazine or streptomycin survived longer than the controls, but were not cured of the infection. A clinical trial with penicillin and sulfadiazine and with atabrine was without effect in a patient reported by Goldberg (15); and the two strains studied by Daniel et al (17) were resistant to penicillin and to streptomycin at 1000 u. per cc. On the other hand Hamilton and Thompson (31) studied the case of a negro boy of 7 with cryptococcus meningitis, to whom penicillin was administered intramuscularly (100,000 u. a day) and intrathecally (20,000 u. a day) for 14 days with a pronounced drop in the number of organisms recovered from the spinal fluid. The course of the infection, however, was not altered, as the treatment may have been begun too late and the dosage of

penicillin may have been too small. This strain was found to be sensitive to penicillin in vitro, indicating that such therapy should be tried in any similar instance, though the reports mentioned above and our own results indicate that this must be a most unusual situation. This report stresses the necessity of attempts at earlier diagnosis and treatment with larger intramuscular doses of penicillin, particularly when the strain's sensitivity has been proved.

Reports have appeared more recently on the isolation and purification of an antibiotic called Actidione (30). This substance was obtained from cultures of Streptomyces griseus, the same strain from which streptomycin is obtained, by different methods of isolation and purification. Because of its marked activity against various fungi—mainly those pathogenic for plants, but also including the Cryptococcus neoformans—, and because of its relative low toxicity for animals in the limited observations so far reported, actidione seems worthy of further evaluation from an experimental and clinical standpoint. A few isolated observations of its use in various fungus infections in man[1] indicate its possible value and apparent lack of toxicity in small doses, and also suggests that it may prove of some help in cryptococcus infections, though there is very little information to go on as yet.

II. CASE REPORTS

Case 1: E.L. #449289: This patient is a 39 year old white salesman of bakery products who was admitted to the Johns Hopkins Hospital on the Orthopedic Service of Dr. Robert W. Johnson, Jr., on January 25th, 1948, complaining of a painful swelling over the sternum for 8 months. His general health had always been good, except for an attack diagnosed streptococcal pneumonia at the age of 18. This was an acute infection involving the right lower lobe and accompanied by pleurisy, lasting about 3 months. The possibility of tuberculosis was considered and X-rays of the chest were taken every 6 months afterward for 7 years, evidently with essentially negative findings. Tonsillectomy was performed 7 years ago. A small, non-painful tumor was removed from in front of the right ear in 1944, which was diagnosed as tuberculosis. This section was reviewed again by the pathologist in Jamestown, New York, and it was considered that it had more the appearance of Boeck's sarcoid than of tuberculosis. The same microscopic slide was examined in September 1949 by Dr. R. H. Follis of the Pathology Department of the Johns Hopkins Hospital. The report of this examination was as follows: "The section is that of a lymph node and about it there are some mucous glands. The architecture

[1] Clinical Reports to the Upjohn Company.

is replaced by collections of epithelioid cells, most of which are made up into discrete nodules and in places such nodules are surrounded by connective tissue. No giant cells and no caseation seen. From the appearance, am unable to make the diagnosis of any specific disease. It is a granuloma whose appearance is compatible with sarcoid, but is not typical. It does not have the appearance of tuberculosis and no cryptococci are seen." (Fig. I.)

Present Illness: Eight or nine months before admission he developed pain in the left anterior wall of the thorax which was aggravated by effort. An electrocardio-

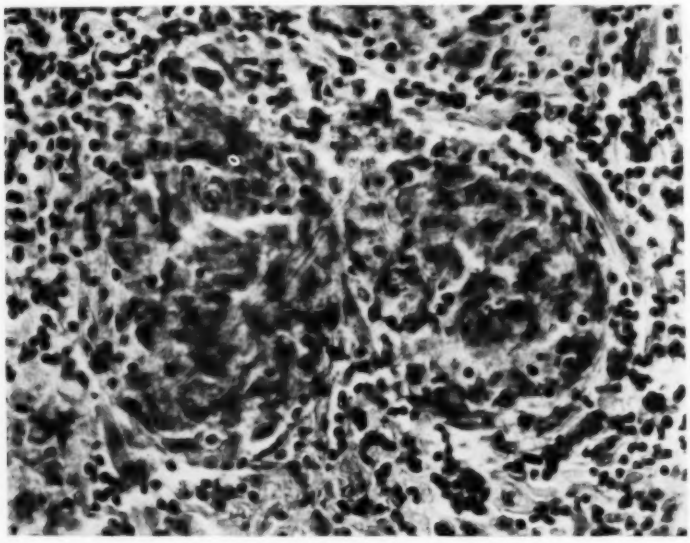

FIG. I. Showing epithelioid cell tubercles in pre-auricular lymph node removed from Case I four years before his admission. Mag. × 300.

gram was taken then and again later, and each time was read as normal. In July and August 1947 the pain was more severe and radiated down the left arm to the elbow. At that time he was thought to have "intercostal neuritis," but soon afterward he had a chill and fever, which was followed by pain in the region of the right hip which prevented him from straightening that leg for several days. This latter symptom gradually improved, though he continued to have some pain in the same region throughout the present illness. He then developed a painful swelling over the lower sternal region, which became progressively larger and which assumed the form of an abscess. This was thought to be tuberculous and was aspirated 5 weeks before admission. Smears of the pus obtained from this abscess revealed no bacteria but yeast forms were found and the diagnosis was thought to be blasto-

mycosis. This lesion was aspirated twice more and at the last aspiration 0.5 gm. of streptomycin was introduced locally. He was given potassium iodide for 4 weeks before admisssion, the dose being increased to 27 drops three times a day with no apparent effect on the lesion.

He was admitted to the Johns Hopkins Hospital on January 25th, 1948. On admission the temperature was 99.6° by mouth and he appeared to be in good health. A scar was found just in front of the right ear, but there was no swelling nor redness there. There was a swelling over the mid-sternal region which was red and fluctuant in the center and indurated at the edges; there was tenderness just to the left of it, along the 4th rib for a distance of about 2 inches. The tip of the spleen was just palpable. There was a dull pain in the region of the right hip on standing, but no abnormality of the extremities was noted. The routine laboratory tests were all normal. An X-ray of the chest showed thickening of the pleura at the right base with some elevation of the diaphragm on that side. No definite evidence of osteomyelitis was made out in X-rays of the sternum and ribs, while a large area of radiolucency was found in the right iliac bone.

The sternal abscess was aspirated and about 10 cc. of thick, milky pus obtained. Yeast-like forms were found in this pus and the organism was identified as Cryptococcus neoformans (Torula histolytica). The right iliac region was next explored by Dr. Johnson and an abscess was encountered which involved that bone. This lesion was thoroughly curetted and a biopsy taken of its wall. Gauze packs soaked with 3½ percent iodine were placed in this area and left in for a few minutes; they were then removed, allowing the excess iodine to remain in the abscess cavity. Pure cultures of cryptococcus again were obtained from this pus and the pathological report was "granulation tissue" which contained numerous giant cells but no tubercles.

The patient recovered rapidly from this operation and the wound healed per primum. The swelling over the sternum did not recur, and he left the hospital nine days after the operation much improved. He was given Lugol's solution and later potassium iodide while in the hospital. After discharge his local doctors continued this at 20 drops of potassium iodide three times a day and also prescribed inunctions of an iodine compound.

Subsequent course: He was seen again on March 23rd, 1948 or seven weeks postoperatively. At this time his temperature was normal and the sternal lesion had cleared up remarkably, leaving no trace of active infection. There was no evidence of active infection in the ilium and the wound was well healed. The spleen tip was still palpable. When last heard from on June 21st, 1949 he reported that the continued to feel well and had no evidence of return of the infection at the above sites. He stated in this letter that he took the iodide and used inunctions of "phenacyle iodide" intermittently for rather frequent pain in the hips, thighs and the right ankle. Dr. Frank Goodwin reported on July 22nd, 1949 that the patient had shown no recurrence to date of the cryptococcus infection, but that recently he had developed an infected plantar wart of one heel which had been removed surgically; the pus obtained and the tissue showed no evidence of cryptococcus infection.

In *summary* this is a man of 39 whose infection was of the localized type, involving a pelvic bone and the sternal region. It seems possible that the portal of entry or at least the initial lesion may have been the granulomatous lymph node which was excised from in front of the right ear 4 years before. It is remarkable that this patient has shown such good results to date from curettage of the bone lesion, aspiration of the sternal abscess and the administration of iodides. This case illustrates well the tendency for infections due to the cryptococcus to remain localized and even latent for long periods of time and the necessity of prolonged periods of observation before one can feel certain of a cure.[2]

Case 2: A.C.: #294622: On March 17th, 1948 this 20 year old unmarried colored female was admitted to the Johns Hopkins Hospital for the second time. On this occasion she complained of swelling of the ankles and pain in the right leg. A sister died 3 years before at a tuberculosis sanatorium; a cousin had tuberculosis. Her general health had always been good. Two and one-half years before this admission she noticed easy fatigueability and mild exertional dyspnea. Sixteen months before admission she developed anorexia, nausea and vomiting. At this time the menses became scanty and then stopped. Two months later she noted loss of axillary and pubic hair, ankle oedema and dryness of the skin. Her first admission to the Johns Hopkins Hospital was 9 months before this one. At that time the diagnoses of Hypopituitarism and Boeck's Sarcoid were established. A lymph node biopsy showed typical changes of the latter condition including many Schaumann bodies. The sarcoid was thought to involve the lungs—with widespread parenchymal infiltration and hilar node enlargement—, the liver, spleen, lymph nodes and probably the pituitary also. There were some unusual laboratory findings, including a low serum calcium (7.6 mgs. %), total proteins of 4.3 gms. %—with albumin 1.7 and globulin 2.6—and serum alkaline phosphatase activity of 34.2 Bodansky units. During most of her stay of 3 weeks she had an irregular daily elevation of temperature to 100° or 101° F.

Three months before the second admission she complained of pain in the right lower leg and was again found to have ankle oedema. One month before admission she developed swelling and more severe pain in the right lower leg and pain also in the lumbar region. At that time a localized area of heat and redness was noted on the right shin and she was found to have generalized oedema. These symptoms became more marked and she was admitted for further study. At that time findings consistent with the diagnosis of Simmonds' disease were again noted, and in addition there was an acutely tender, fluctuant swelling over the surface of the right tibia, a moderate general glandular enlargement, several small subcutaneous nodules, tenderness over the left iliac crest, ascites, an enlarged liver and ankle oedema. Condylomatous lesions were noted on the vulva—having been present for several months. X-rays revealed destructive lesions in the right tibia and left

[2] He was reexamined on March 4th, 1950. No evidence of any cryptococcus infection was found; the sternal and iliac lesions being entirely healed, both clinically and by X-ray.

ilium, and biopsy of the tibial swelling showed a granulomatous type of tissue in which numerous giant cells were found, many of which contained large organisms surrounded by a clear zone. These were thought to be cryptococci. (Fig. II.) At the operation grayish pus was encountered which on smear was found to contain numerous yeast-like organisms. From this pus a pure culture of yeast-like forms was obtained which were thought to be either blastomyces or cryptococci and which were later identified as Cryptococcus neoformans. The same organisms were later found in a biopsy of a subcutaneous nodule, in cultures from the perineal

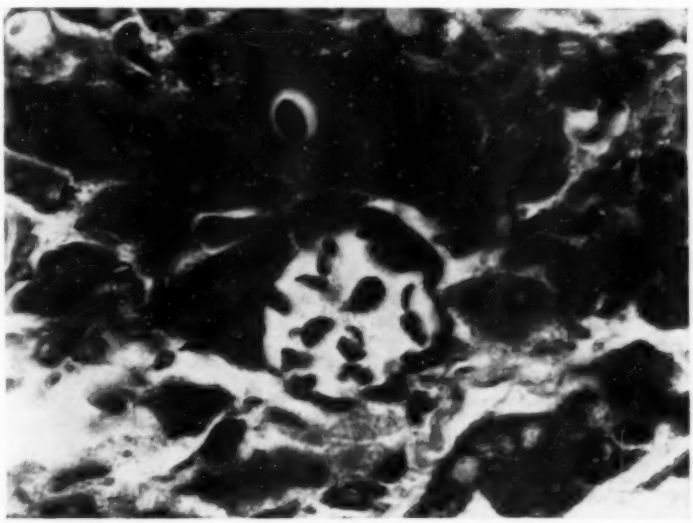

FIG. II. Granulation tissue from bone lesion of Case II. This illustrates the frequent tendency for the organisms to grow in cyst-like collections in the tissues. In other areas in this section cryptococci are found within phagocytic cells. Mag. × 1200.

region where ulcerative purulent lesions developed, and from an abscess which appeared over the left ischium. Laboratory tests again showed a very high level of serum alkaline phosphatase (52.3 Bodansky units), low total proteins and a serum calcium of 8.2 mgs. %. The bromsulphalein test indicated some hepatic impairment, the cephalin flocculation test was one plus, and the thymol turbidity 5.6 units. X-ray of the pelvis showed extension of a destructive lesion which had previously been found in the left ischium. Lumbar puncture one month before death revealed clear fluid containing 45 mononuclear cells per cmm., and protein of 106 mgs. %. Three weeks later the fluid contained the same number of cells,

protein of 150 mgs. % and sugar 9 mgs. %. No organisms were grown from these fluids, but outspoken signs of meningitis developed soon after, and a heavy growth of cryptococcus was obtained from spinal fluid removed on the day of death (July 5th, 1948). This was 3¼ months after her admission.

The progress of the infection, therefore, in this patient who was suffering simultaneously with widespread sarcoidosis, was steadily progressive and apparently uninfluenced by the various therapeutic measures attempted. This therapy included potassium iodide, penicillin and sulfadiazine—all in large doses.

It was the clinical impression that the patient had sarcoidosis with involvement of the lymph nodes, lungs, pituitary and liver, and torulosis of the meninges, brain, tibia and ischium.

At autopsy, which was performed by Mr. J. G. Wells under the supervision of Dr. M. Berthrong, the following anatomical diagnoses were made: *Sarcoidosis* of lungs, heart, lymph nodes (generalized), liver, spleen and hypophysis. *Simmonds'* disease. Cachexia. Atrophy of breast, ovary, uterus, (?) thyroid, adrenal cortex and loss of pubic and axillary hair. Ascites. *Cryptococcosis* (C. hominis) of meninges, ependyma, brain, kidneys, adrenal, lung, bone, skin and subcutaneous tissues. Congestive splenomegaly. Lobular pneumonia. Hydrothorax. Primary tuberculous complex, lung. Lymphangioma, ovary.

Case 3[3]: M.L.: #503842: This was a 7 year old white farm boy who was admitted to the Neurosurgical Service of the Johns Hopkins Hospital on June 12th, 1949, complaining of severe headache and nausea for 6 weeks. The patient's past health had always been good with the exception of pneumonia at 3, a tendency to frequent colds and repeated attacks of "sinusitis." Six weeks before admission he developed a severe frontal headache, nausea and vomiting. These symptoms progressed in severity with almost daily vomiting and occasional feverishness. During the week before admission the neck became stiff.

The admission findings were temperature 101.6° F., pulse 88 and respirations 20 per minute. He was acutely ill, dehydrated and showed evidence of weight loss. He held his hand on his forehead continually, complaining of severe headache. There was no evidence of infection of sinuses or ears. There was a moderate degree of papilloedema, a stiff neck and a weakly positive Kernig sign. The urine examination was negative and the white blood count 9,800 per cu. mm. The most likely diagnosis seemed to be either a brain tumor, probably in the posterior fossa, or meningitis of some sort. A careful lumbar puncture was done by Dr. Robert Green. The fluid which was obtained was under a moderate increase in pressure; the protein was elevated and the sugar depressed to 23 mgs. %. There were no blood cells but 120 cells per cu. mm. were found which had the appearance of yeasts. Most of these cells had buds and an India Ink preparation showed them to have capsules;

[3] I should like to thank Dr. A. Earl Walker, Professor of Neurological Surgery, for his permission to include a summary of this patient's record. The case is being reported in more detail, from a clinical and pathological standpoint, by Dr. R. E. Green (35).

on the basis of this the diagnosis of torula (cryptococcus) meningitis was made and this was verified by cultures. Lumbar punctures or ventricular taps were done repeatedly on subsequent days, the chief findings being a low sugar level (5–12 mgs. %), few w.b.c. (0–10 per cmm.) and a cryptococcus count at one time as high as 198, and on two occasions zero. Inasmuch as this strain was found to be resistant in vitro to other antibiotics but sensitive to actidione, and because actidione has not proven toxic to human beings when given in small doses (32), it was administered to this patient both intramuscularly and later intraventricularly. This therapy was tried after negative results had been obtained with penicillin and with sodium bicarbonate.* The intramuscular actidione was given in doses of 20 mgs. per day for 9 days, with no evidence of improvement nor of toxicity. During this treatment the patient's blood serum showed only slight inhibition of the cryptococcus in vitro. The same substance was administered intraventricularly in amounts of 1 mg., 1 mg., 2 mgs., 5 mgs. and 10 mgs. on successive days. During this period the count of the yeast cells dropped from 76 to 10 per cu. mm., and 2 and 3 days later to zero. Cultures also showed a striking drop in the number of organisms. However the patient showed no clinical evidence of improvement and this therapy was abandoned. Supportive treatment was used during the rest of his course, which was marked by increasing intracranial pressure, temperature of 100° to 102° F., coma and finally signs of decerebrate rigidity. A ventricular tap done the day before death showed 140 cryptococci. He died on July 29th, 1949—approximately 7 weeks after admission or 3 months after the onset of infection. The clinical diagnosis was Torula meningitis (or meningoencephalitis).

Autopsy was done by Dr. Gordon R. Hennigar and the following anatomical diagnosis established: Cryptococcus meningo-encephalitis. Cryptococcus lesion in kidney. Pulmonary oedema. Some of the outstanding microscopic features noted by Dr. Hennigar were the following: Necrosis was quite marked and there was an abundant mononuclear response. Organisms were seen in great numbers in the choroid plexus and in foreign body giant cells. The ependyma and subependyma regions throughout were involved; in some places the inflammatory reaction was diffuse, while in others focally granulomatous. . . . Focal, tiny granulomata were seen in the white matter of the cerebrum and cerebellum. A small cryptococcus lesion was seen in one kidney.

III. EXPERIMENTAL STUDIES

(1) *Isolation* and *Identification*

Strain I: The organism was recovered from the sternal and ilial abscesses in the first patient. It grew equally well at room or incubator temperature (37° C), and produced a light growth on blood agar and in Brewer's thioglycollate medium in 2 days. On Sabouraud's medium

*Described more fully in another report (35).

there was a good growth in 2 days of grayish, cream-colored colonies that became orange and finally brownish in color. The colonies had a slightly moist character but were never as "slimy" in appearance as those from the second case (Fig. III). No mycelia were ever found. Microscopically the organisms were yeast-like in appearance with a tendency to form single buds; they were ovoid and round and sur-

FIG. III. Cultures of the three strains on Sabouraud's medium. The slimy appearance of Strain II is illustrated, in contrast to the somewhat pasty character of the others.

rounded by a capsule. This capsule was usually a small one (in contrast to that of the second case), though an occasional yeast had a larger capsule. It was found to ferment sucrose and dextrose slowly, but not mannite, lactose or maltose. A culture was sent to Dr. Norman Conant at Duke University who identified it as *Cryptococcus neoformans*.

Strain II: Pus was obtained from an abscess of the tibia. A prepa-

ration of this with dilute India Ink revealed yeast-like organisms with large capsules. Growth was obtained as in the other strain on the routine media at room and incubator temperature and the best growth was obtained on Sabouraud's medium. On this the colonies appeared at first to be moist and cream colored; 2 or 3 days later the colonies were confluent, orange color and very slimy in character. The color later became brown, the growth retained its moist character, and was found to be quite stringy when removed with a platinum loop. Smears of all cultures of this strain showed that it always retained its capacity to form large capsules. Dr. Conant again was kind enough to study this strain for us and, likewise, identified it as *Cryptococcus neoformans.*

Strain III: This was isolated from the spinal fluid of the third patient, an India Ink preparation revealing buds and capsules. Its cultural characteristics were identical with the other two strains, a vigorous growth appearing on Sabouraud's medium in 2 days. The initial growth had the same color and moist appearance as the others, and became brownish after a few days. However, the older colonies soon lost their moist character and seemed drier and more pasty than either of the others. Moreover, the capsules on this strain were definitely smaller than those of the other two, though it is of interest that all three were equally pathogenic for mice, as will be shown later.

(2) *In Vitro Inhibition Tests*

(a) *Sulfonamides:* Strain I: Weak inhibition by sulfadiazine at 25 mgs. % and by sulfamerazine by 50 mgs. %. Strain II: Complete inhibition by sulfadiazine at 500 mgs. % but only slight effect at 250 mgs. %. Good but not complete inhibition by sulfamerazine at 500 mgs. % and a weak effect at 250 mgs. %.

(b) *Iodine:* Strain I: Inhibited or killed by Lugol's solution diluted 1:40 and by tincture of iodine (3½%) at 1:80.

(c) *Penicillin:* Strain I: *No* inhibition from 500 units to 8 units per cc. (In broth and in Brewer's thioglycollate medium) with either heavy or light inocula.

Strains II and III: *No* inhibition from 250 units to 8 units per cc. (in same media and with same inocula).

(d) *Streptomycin:* Strains I, II and III: *No* inhibition from 250 to 2.5 micrograms per cc.

(e) *Propamidine:*[4] (Merck) (or 4:4′-diamidinodiphenoxypropane): Strain I killed by 0.062% in dextrose broth, and inhibited by dilutions as low as 0.008%. Strain II: Killed by 0.25% and inhibited by 0.008% in the same medium.

(f) *Aureomycin:* Strains I, II and III: *No* effect from 500 to 8 gamma per cc.

(g) *Chloromycetin* (Chloramphenicol): Strains I, II and III: *No* effect from 500 to 2 gamma per cc.

(h) *Actidione:* Strain I: Killed by 0.002 mg., Strain II: Killed by 0.001 mg., Strain III: Killed by 0.008 mg. per cc.

(3) *Mouse Virulence Tests*

Since the intravenous route resulted in a more uniform course with a higher mortality than that following other types of inoculation, this route was employed in these experiments. The tail vein was used and the inocula were saline suspensions of the cryptococcus cultures taken from Sabouraud's slants at various periods of growth and given in various dilutions. In one such experiment approximately 8,000,000 organisms of a 3 day growth of *Strain I* resulted in death of 2 mice in 8 and 9 days and the cryptococci were recovered in pure culture from the heart's blood, lung, liver and spleen. In 2 other mice larger inocula of this strain resulted in death in 4 days with similar cultural findings in the organs, including the brain. *Strain II* in amounts of approximately 16,000,000 organisms of a 2 day culture resulted in death of 3 mice in 5 and 7 days. Pure cultures of the infecting agent were recovered from heart's blood, lungs, liver, kidneys, spleen and brain of all infected animals.

In other experiments attempts were made to determine the relative virulence of the three strains for mice by intravenous administration. The results of these studies are summarized in Table I. In one series 4 large mice were given intravenous inocula of 250,000 organisms of a 3 day growth of *Strain I* and 4 other mice were given 260,000

[4] This drug was obtained at the suggestion and through the kindness of Dr. E. B. Schoenbach of the Department of Preventive Medicine of The Johns Hopkins University. A review by Schoenbach and Greenspan on the properties of this and other Diamidines (34) indicated its possible therapeutic effect on infections due to the cryptococcus.

of a 2 day culture of *Strain II*. Not all mice in either group died during the course of this experiment, though all became sick. The animals were killed at periods varying from 20–47 days and the brain cultures uniformly resulted in a heavy and pure growth of cryptococci. Cultures from other organs and from the heart's blood yielded variable results, though they were more often positive in those mice inoculated with Strain I than with Strain II. These data plus the fact that the

TABLE I

Mouse Virulence Tests

STRAIN AND INOCULUM*	CAPSULES (IN VITRO)	SIZE OF MICE	NUMBER OF MICE	RESULTS			
				Died (or killed when sick) Aver. No. days	Cultural Findings†		
					Hearts' blood	Brain	Other organs
I (250,000)	±	Large	4	26.5	0 to ++	++++	0 to +++
II (260,000)	+++	Large	4	29	0	+++ to ++++	0 to +++
I (42,000)	±	Small	5	12.2	++ to +++	++ to +++	+ to ++++
II (20,000)	+++	Small	5	21.2	+ to +++	+++ to ++++	0 to ++++
III (660,000)	±	Large	4	21.3	+ to ++	++++	+ to ++++

* Number of organisms; given intravenously.

† 0 = no growth. ++++ = heavy, confluent growth on Sabouraud's medium.

Strain I animals all looked sicker than the others suggested that Strain I was possibly a little more virulent for these large mice than was the other.

In a similar experiment small *young* mice were employed and much smaller inocula used, namely 42,000 of Strain I and 20,000 of Strain II. (The age of these cultures was 5 and 4 days respectively). The 5 animals receiving Strain I died in 10–15 days, with an average of 12.2 days, and cultures of heart's blood, lung, liver, kidney and brain were all strongly positive for cryptococci. The 5 mice receiving the Strain II culture died or were killed when quite sick in 17–26 days with an

average of 21.2 days. In each of these animals brain cultures revealed a heavy growth of organisms, while cultures from other organs were less consistently or strongly positive than were those of Strain I.

These results would therefore indicate that Strain I was at least as virulent for mice as was Strain II, though a smaller inoculum was employed for the young mice receiving the Strain II cryptococci. This was a somewhat unexpected finding in that the former was isolated from the patient who had localized lesions, whereas the latter was from the case with the generalized form of the disease. Moreover, the Strain I cryptococci had much smaller capsules and produced a relatively rough type of colony growth, whereas the other strain had large capsules and regularly produced a smooth, gelatinous type of colony growth on laboratory media—findings usually associated with increased virulence, at least in bacterial species. This property, however, does not hold true in the tissues of the host, where the relatively non-encapsulated strains have been found to produce large capsules (Fig. IV). In another similar test with Strain III using 4 large mice and an inoculum of 660,000 organisms the virulence appeared to be of essentially the same magnitude though, of course, definite conclusions cannot be drawn from experiments in which so few animals are employed.

In all of these mice, as in those to be reported in the following therapeutic attempts, the cultural findings were roughly similar. Cultures taken directly from the brain almost invariably showed a heavy, confluent type of growth, which is indicative of the marked affinity of the cryptococcus for this tissue. A loop-ful of the heart's blood was the standard inoculum from that organ, and in some it showed no growth, though there were usually a few colonies. The lung cultures were taken from the cut surface of one lung and were usually graded 1 plus to 2 plus (or approximately 1 to 20 colonies). The liver and kidney cultures were made from their cut surfaces and the grading was 2 plus to 3 plus in most instances. Because of these fairly constant and predictable findings it seemed that the mouse was a satisfactory animal to use for a study of various therapeutic agents.

(4) *Therapeutic Tests*

Mice were employed in the therapeutic trials, though the criticism might be offered that these animals are not entirely satisfactory inas-

much as their susceptibility to the Cryptococcus neoformans is of such a high order, and one may not be justified in transferring the results of such experiments directly to humans. However, it would seem that a positive result in this species might for the same reason be of real

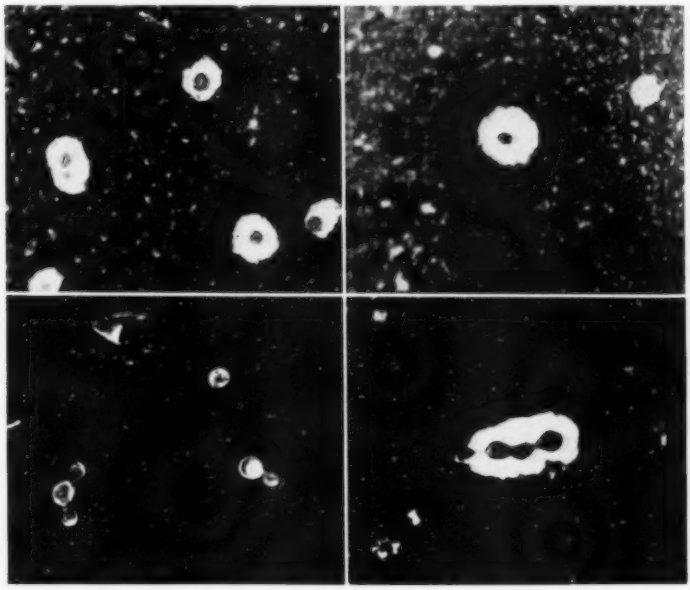

FIG. IV.: *Cryptococcus Capsule Studies.** (1.) upper left: Strain II—Three day growth on Sabouraud's medium. Note production of large capsules by this strain even on artificial medium. (2.) upper right: Strain II—From mouse peritoneum. In vivo the capsules tend to be even larger. (3.) lower left: Strain III—From Sabouraud's medium. Almost no capsular substance visible. (4). lower right: Strain III—From mouse brain. Large capsules appear after inoculation into mice. (Similar studies done with Strain I also showed absence of capsules in vitro and large ones in vivo.) Mag. × 1200.

significance; consequently, an attempt was made to influence the course of the experimental infection in mice with various agents, some of which gave promise of therapeutic activity in vitro, whereas others did not.

* India Ink preparations counterstained 24 hours with Saffranin.

METHODS

Inocula of varying strengths and ages were given intravenously in amounts which had been shown to result in an established infection or death in mice. The therapeutic agent under investigation was usually administered either immediately or within 1 or 2 hours after the inoculation and was given (except in the case of sulfadiazine) subcutaneously, intramuscularly or intraperitoneally, once or twice daily and in varying amounts. Controls were run with each individual study and results calculated on the basis of death or survival of the mice and on cultures obtained from the mice which died or were killed at the end of the experiment. It may be said at the outset that the results were all "negative" but it seemed worth while to give some of the details, which might prove of some value to others making a similar study of this unusually resistant type of micro-organism. The results are summarized in Table II.

(a) *Propamidine:* In vitro tests showed this to possess definite anti-cryptococcal activity as mentioned earlier. It was found that large mice could tolerate a dose of 0.5 mg. per day i.m. for 7 days.

One group of 4 mice was given this drug in doses of 0.2 mg. b.d. (i.m.), another 4 received 0.5 mg. in one dose per day and 3 mice served as controls. The injections were begun 2 hours after a large infecting dose of Strain II. The treated animals all died in 3 days, the control in 9, indicating toxicity of the drug at these dosage levels. Positive cultures from the organs indicated that the cryptococci were not killed in this experiment though the duration may have been too short. Another test was done using much smaller inocula (80,000 organisms of Strain II) and a smaller dose of propamidine (0.1 mg. b.d.). The results of this likewise seemed entirely negative.

(b) *Aureomycin:* In spite of its lack of in vitro activity an in vivo test was done with aureomycin, in which 1,600,000 cryptococci of Strain II were given i.v. and the antibiotic given in doses of 0.5 mg. per day in one dose intraperitoneally. Two mice were used and no inhibiting effect noted.

(c) *Penicillin:* It seemed worth while to test the effect of this antibiotic in mice, because of the possibility that the in vivo activity might not parallel the in vitro. Moreover, in one series of tubes, marked inhibition of Strain II was noted at a level of 31 u. penicillin per cc.,

whereas little or none was found at higher or lower levels. This isolated finding, which suggested a possible "optimum" concentration of

TABLE II

Therapeutic Trials in Mice

STRAIN AND INOCULUM	NUMBER OF MICE	THERAPEUTIC AGENTS*	RESULTS			
			Died (or killed) Aver. No. days	Cultural Findings		
				Hearts' blood	Brain	Other organs
II (80,000)	3	Propamidine† 0.2 mg.	D 14.3	0 to +	+ to ++++	0 to +++
II (1,600,000)	2	Aureomycin 0.5 mg.	K 7	+++	+++	+++
II (3,200,000)	2	Penicillin (Procaine) "Crysticillin" 2,000 u.	D 6	++	+++	++ to +++
	4	20,000 u.	D 5	0 to ++	++ to ++++	+ to +++
II (32,000)	4	"Depo-Penicillin" 2,000 u.	K 18	0 to +	+++	0 to +++
	4	20,000 u.	K 18	0 to ++	+++ to ++++	0 to +++
II (90,000)	3	Actidione 1 mg.	D 12	+	+ to ++++	+ to ++++
	4	0.2 mg.	D(3)‡ 10. K(1) 16	0 to ++	+ to ++++	0 to +++
III (660,000)	5	1 mg.	D(3) 15. K(2) 18	0 to +	+++ to ++++	0 to +++
	5¶	0.2 mg.	K 22	+ to ++	++++	+ to +++
I (68,000)		Sulfadiazine (% in food & aver. mean intake per day)				
	4	1% 34 mg.	D(2) 13. K(2) 13.5	0 to +	++ to +++	0 to +++
	4	0.5% 23 mg.	K 17.5	0 to +	+++ to ++++	0 to ++

* Total dosage per day.

† Earlier death in mice receiving larger doses of Propamidine described in text.

‡ Numbers in parentheses refer to numbers of mice.

¶ One animal in this group was not sick when killed, and cultures were all sterile. Error in inoculation?

penicillin however, was never found in subsequent tests with this or the other strains and in various media. In one series of experiments

large inocula (3,200,000 organisms of a 4 day growth of Strain II) were given to 11 mice. Four of these were treated with procaine penicillin ("Crysticillin"—Squibb) 10,000 u. subcutaneously b.d., 4 more were given 1,000 u. b.d. and 3 were controls. These mice all died in 3 to 11 days, and practically all cultures were positive and to essentially and equal degree.

Because the inocula were so large and also because of the difficulty of guaging the exact dosage of penicillin when this preparation is used diluted in saline—it having a tendency to precipitate rapidly in the syringe before injection—it was decided to repeat this experiment under slightly different conditions. Consequently, 32,000 organisms of the same strain and same period of growth as the above were given to 11 mice (weighing approximately 15–20 grams). Four of these were given 20,000 u. of Crystalline Procaine-penicillin in peanut oil with aluminum monostearate ("Depo-Penicillin"—Upjohn) i.m. or subcutaneously once a day. Four others were given 2,000 u. a day and 3 were controls. These mice died or were killed when obviously sick in 6 to 21 days and the organ cultures were equally positive in the treated animals and in the controls.

(d) *Actidione:*[5] The in vitro activity of this antibiotic for the pathogenic strains of cryptococci has been mentioned earlier. To recapitulate, Strain II was killed by 0.001 mg., Strain I by 0.002 mg. and Strain III by 0.008 mg. per cc. Its anti-cryptococcal activity against this latter strain was not altered by the patient's serum. Some in vivo activity of the actidione was noted in this patient also, but the course of his illness was not noticeably altered by its use. In mice two strains were tested (II and III) after it was shown that mice were killed in 5 days by 2 mgs. per day (100–150 mgs. per kilo) and by 1 mg. per day (50–75 mgs. per kilo) in 8–12 days. They tolerated 0.1 mg. and 0.2 mg. per day without signs of toxicity, though pathological studies were not done.

In the first test 11 mice were given 90,000 organisms of a 2 day growth of Strain II. Eight of these mice were large and 3 small (one in each group). The first group of 4 was given 1 mg. actidione (i.m. or s.c.) per day; the second 4 received 0.2 mg. per day and the other 3

[5] This drug was very generously supplied to us by The Upjohn Company for experimental and clinical trials.

were controls. All these animals died in the usual time and the cultures taken from the organs of the treated ones did not differ significantly from the controls.

A similar experiment was run with Strain III using 660,000 of a 2 day culture of the cryptococcus. Treatment was begun 24 hours later with actidione. Five mice were given 0.2 mg. per day (i.m. or s.c.) and 5 others 1 mg. per day. Large mice were used. The mice were treated for 19 days, the drug being given twice a day for 5 days and once a day for 14 days. The ones receiving the larger doses showed toxic effects of the drug for they all were sicker than the controls and 3 died in 10–14 days. One of those receiving the smaller dosage did not look sick when killed after 24 days and cultures were all negative for cryptococci. (This may represent a mistake in inoculation, but should be repeated). All the other treated and control animals were killed after 19 to 23 days and all the usual cultures were positive in varying but equal degrees, with the brain cultures showing heavy (4 plus) growth.

This experiment, therefore, may likewise be considered essentially negative for any effect of actidione on cryptococcal infection in mice.[6] However one of the animals treated with the smaller doses was found to be free of the infection at autopsy, as determined by cultures of all organs, though histological studies were not done.

(e) *Sulfadiazine:* Each of 10 mice were inoculated intravenously with 68,000 cryptococci of Strain I. Feeding experiments were done according to the methods outlined by Marshall et al (33). In these tests individual cages were used; the sulfadiazine was incorporated in finely ground purina dog chow, mixing thoroughly so as to produce concentrations of 1% and 0.5% of the drug in the diet. Four mice were fed the 1% mixture, 4 the 0.5% and 2 were controls. Ten per cent was allowed off for food that was spilled and could not be recovered. The 1% mixture was not eaten as well as the 0.5% which was taken nearly as well as the control diet. Two of the mice on the 1% diet died in 7 or 8 days; the rest were killed at the end of 16 to

[6] Negative results were likewise obtained by F. R. Heilman of The Mayo Clinic in experiments with mice inoculated intraperitoneally with cryptococci and treated with actidione. This series of tests was reported to The Upjohn Company in April 1948.

19 days, at which time they all appeared sick. The mean drug intake for the animals on the 1% sulfadiazine was calculated at 29, 42, 39 and 27 mgs. per day. That of those on the 0.5% diet was 18, 24, 26 and 23 mgs. per day. All these amounts should produce high blood levels, as judged by the figures of the other workers for sulfanilamide, and according to a personal communication to that effect by Marshall. Cultures were taken from the organs of the animals in the usual way and showed that in each animal there was heavy growth of the cultures from the brain, indicating that the infection was not eradicated or even held in check in any instance. In only one animal was the brain culture significantly more weakly positive than were similar cultures from the controls, but in several mice the cultures from other organs were sterile while the controls were weakly positive. This would indicate therefore that the sulfadiazine may have exerted slight inhibition on this strain of cryptococcus in mice, though it certainly was not marked enough to be of any real significance.

DISCUSSION

The data considered in this report indicate that Cryptococcosis (or torulosis) is a rare condition as shown by the fact that to date only about 120 cases have been recorded in the literature. This impression is further strengthened by the finding of only four other cases in the pathology files of the Johns Hopkins Hospital during a period of twenty years from 1928–1948. In one of these cases the meninges alone were involved, in another there was meningitis and torula infection of the prostate, while in the other two the infection was generalized, with involvement also of the brain and meninges.

The three cases here recorded are of especial interest in that they illustrate most of the features which have been observed in other cases of the disease as reported in the literature. Case I demonstrates the tendency in some instances for the infection to produce localized lesions with few constitutional symptoms. In this patient the primary lesion may have been a preauricular lymph node, though no cryptococci were actually found in the section. In him the disease has apparently been quiescent or cured for 2 years, but a much longer period of observation is necessary before we can feel confident that the infectious agent has been eradicated from his tissues. In Case II the

infection was generalized and complicated by sarcoidosis, an association that has not been reported previously. This does not suggest any reciprocal relation between the two diseases but seems rather to point to the fact that a patient with a chronic, debilitating disease may be more prone to develop cryptococcosis than would an otherwise healthy person. This may be analagous to the situation in Hodgkin's disease which is known to occur with relative frequency in association with cryptococcus infections (10,11). No data were obtained clinically or pathologically which would help explain the portal of entry in this patient. In Case III the meningo-encephalitis was apparently "primary", or at least uncomplicated by any other evidence of cryptococcal infection. In this type of case one wonders if the organisms may not gain entry into the body by way of the upper respiratory tract and then either by direct extension or by the blood stream into the central nervous system for which they have an especial affinity. One wonders also in this boy whether or not the history of "sinusitis" may have been of significance in respect to a possible primary infection. It is interesting also to find that in two of these three cases bone lesions were among the most prominent findings, an occurrence that is considered uncommon in cryptococcus infections.

As was mentioned previously, a constant feature of this infection is the tendency for the organisms to incite only a low-grade local inflammatory reaction. This may, in part, account for the tendency of the organisms to remain in the tissues of the host for long periods, being relatively uninfluenced by the phagocytic powers of the blood and tissue cells, though they are phagocytized to some extent by mononuclears and giant cells. The large amount of capsular substance possessed by the cryptococci may be of especial significance in this respect, as is the case with most bacteria, in which encapsulation is associated with increased virulence. In the three strains of cryptococci under consideration in this study, though two of them were found to contain very little capsular material when grown on artificial media, all of them produced large capsules in animal or human tissues. Likewise, in preparations made from infected mouse tissues, one regularly finds that there is a wide, clear zone outside the capsule itself. This zone is made up of "extra-capsular" material which appears clear when stained for short times, but can be stained irregularly and meta-

chromatically with longer periods of staining. These features are illustrated in Figs. V and VI.

The experimental studies emphasize the high degree of susceptibility of the mouse to this infection, all three strains being equally potent

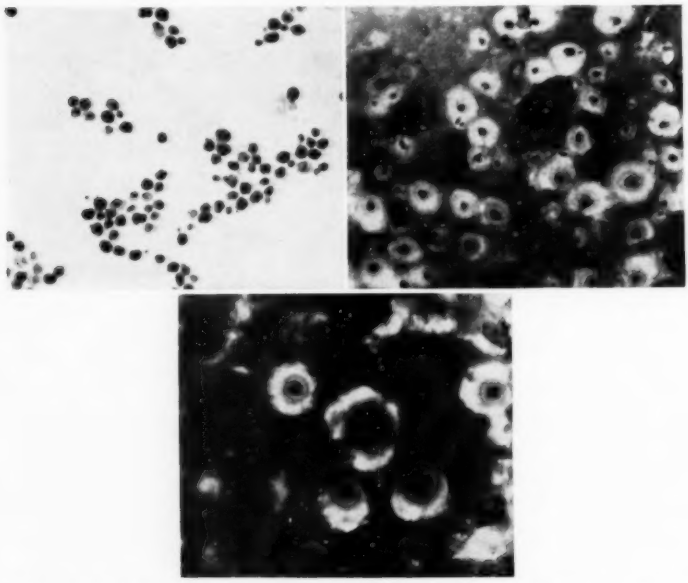

FIG. V: *Staining Properties of Cryptococci and Their Capsules.** Upper left. Strain I—Culture from Sabouraud's medium. No capsules visible. Upper right. Strain I—Smear from brain of mouse inoculated intravenously. One notes sharply outlined capsules around the organisms and larger zones of extra-capsular substance. No apparent phagocytosis in this field. Lower. Strain II—Smear from brain of mouse inoculated intravenously. Appearance much like that of B. Some of the extra-capsular substance has become stained. This gives a metachromatic stain, being pinkish-purple in contrast to the deep blue of the cryptococcus itself. Mag. × 1200.

in this regard, as small a dose as 50,000 cryptococci intravenously usually being enough to kill young animals in 2–3 weeks. Subcultures in such cases show that—as in man—there is a strong predilection for

* Stained with Methylene Blue (1/2%) for 30 minutes.

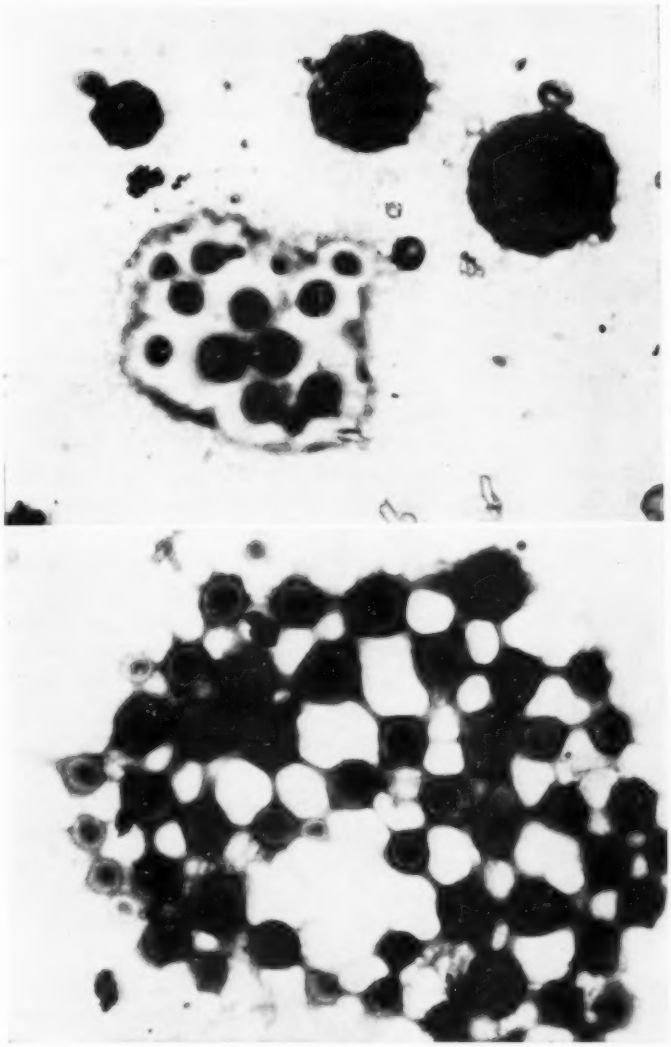

FIG. VI. *Some Characteristics of the Extra-Capsular Substance.* Upper. Strain I—From infection in peritoneal cavity of mouse. Stained 10 minutes with Toluidine Blue (0.01%). One notes the tendency for the cryptococci to grow into cyst-like groups which contain the clear extra-capsular material and are sharply delineated by a blue-staining membrane. (A phagocyte is seen which contains two cryptococci.) Lower. Strain II—From infection in peritoneal cavity of mouse. Stained one hour with Methylene Blue (¼%). Masses of cryptococci are seen; the capsules stain metachromatically. Some of the extra-capsular material is stained by this method and forms bridge-like connections between the organisms. ×1200.

the central nervous system. The mouse, therefore, makes a good test animal, not only for virulence studies but also for therapeutic tests. However, it may be argued that because the mouse is so susceptible to this experimental infection, it does not offer a fair therapeutic test against a species which so often produces a chronic, low-grade infection in man. On the other hand, any drug or antibiotic which even modifies the infection in mice to a significant degree should also be tried on other animals, and deserves careful consideration in cases of the disease in man.

The therapeutic trials recorded in this report—both in mice and in man—have not uncovered any agents which can be considered capable of exerting a definite effect against cryptococci, organisms which produce such a persistent infection once they gain a foot-hold in the tissues of the host. Though the in vitro tests were encouraging with actidione, the in vivo results were very disappointing, and the course of the one patient who received it certainly was not altered by its use. The fact, however, that cultures of the spinal fluid taken before and after its direct introduction into the meninges did show a marked reduction in the number of cryptococci present in the fluid itself, suggests that it probably does have some fungicidal effect in vivo, provided it can be brought into direct contact with the infecting agent. This viewpoint is further strengthened by in vitro tests which showed that the action of actidione was not inhibited by the patient's serum.

Published reports indicate that sulfadiazine may have had a beneficial effect in some instances, though we did not observe any such results from its use in experiments with mice nor in clinical trials in two of our cases. There is, however, considerable variation in its effect on different strains of cryptococci, and each patient should be given an adequate trial of this drug.

One of our cases seems to have been benefited by drainage of the local lesions, direct application of iodine, and the administration of potassium iodide by mouth. Such a regimen seems definitely indicated in any patients in whom localized lesions are found which are amenable to surgical drainage, and the iodides should be given in large doses to the individual tolerance of the patient.

Penicillin seemed to be without effect in 2 of our patients to whom it was given (Cases II and III), and our experimental studies with

all three strains were in agreement with this finding. However, the marked reduction in the number of cryptococci in the meninges, as shown by cultural studies in one patient mentioned earlier (31) who had meningitis and was treated intrathecally with penicillin, suggests that this therapeutic agent be given a thorough trial in the exceptional case in which the strain is found sensitive.

Any chemotherapeutic agent which may be found of real value in dealing with the generalized or the meningo-encephalitic forms of cryptococcus infection will have to be one capable of a high degree of diffusibility, in order to penetrate into the collections of cryptococci which tend to form cyst-like lesions in the tissues. It is probable that the virulence of the cryptococcus is enhanced by its capsule, since this has been found true of most types of bacteria. Therefore it is possible that any substance which would destroy the capsules might render the cryptococci more susceptible to destruction by the host. Such an accomplishment is obviously a difficult one but need not be considered impossible, in spite of the apparent insignificance of the host's immune mechanism, and the inefficiency of his cellular reaction.

SUMMARY

1. This report deals with three cases of cryptococcosis (or torulosis), two of which were fatal.

2. In one the infection was apparently localized; in another it was generalized and associated with widespread sarcoidosis causing Simmonds' disease and severe liver damage; in the third the course was more rapid and the lesions limited to the meninges and brain.

3. In two of the cases bone lesions were prominent features of the illness. This is said to be relatively infrequent in cryptococcus infections.

4. In the first case surgical drainage of a bone lesion and iodide therapy seem to have been of real benefit; in the others no treatment altered the progressive course of the infection.

5. Experiments are reported in which mice were employed for virulence studies and chemotherapeutic tests; all of the latter yielded negative results. The chemotherapeutic agents tried were: Propamidine, Aureomycin, Penicillin, Actidione and Sulfadiazine.

6. It seems likely that the usual progressive course of this infection

to a fatal termination is best explained on the basis of the poor antibody response that has been found in such cases, the protective effect of the abundant capsular and extra-capsular material on the surface of virulent cryptococci and the inefficient phagocytic reactions of the host's cells.

7. Search should be continued for an antibiotic or chemotherapeutic agent capable of modifying or curing this unusually resistant infection.

The photographs were taken by Mr. Chester F. Reather of the photographic department of the Johns Hopkins Hospital and Medical School.

The author would like to thank Dr. G. S. Mirick, Dr. R. W. Johnson, Jr., Dr. G. R. Hennigar, Mr. J. G. Wells, Dr. R. E. Green, Dr. E. B. Schoenbach, and other members of the departments of medicine, orthopedics, neurosurgery and pathology whose help has made this study possible.

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THE EFFECT OF ALUMINUM HYDROXIDE ON SERUM AUREOMYCIN CONCENTRATIONS AFTER SIMULTANEOUS ORAL ADMINISTRATION

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Received for publication March 28, 1950

Aureomycin is readily and completely adsorbed from aqueous solution by aluminum hydroxide, silica gel, charcoal, magnesium oxide and other well known adsorbents (1, 2). It is also adsorbed from serum by silica gel (3). After adsorbtion from aqueous solutions or serum it is exceedingly difficult to remove the aureomycin from the adsorbent. Thus, aureomycin administered with aluminum hydroxide should be bound to the aluminum hydroxide and remain in the gastrointestinal tract. Waisbren and Hueckel measured the blood concentration of patients receiving aureomycin and reported a fall when aluminum hydroxide was given (4). Aluminum hydroxide is frequently prescribed with aureomycin (5, 6) to relieve gastrointestinal symptoms. Experiments to determine quantitatively the effect of administration of aureomycin with and without aluminum hydroxide in normal individuals have been undertaken.

Eleven mg. per kilogram of body weight of aureomycin hydrochloride* in gelatin capsules was orally administered to nine healthy subjects. Serum levels were measured at 2, 4, 8, 12 and 24 hours by adsorbing the aureomycin on silica gel columns and observing fluorescence (3). The same subjects under the same conditions several days later then took 11 mg. per kilogram of aureomycin and 0.3 ml. per kilogram of a commercial USP aluminum hydroxide gel preparation (Cresalgel) and the serum levels were redetermined. All medications were taken at 8:30 A.M. Fasting subjects ate no breakfast but ate lunch between noon and 1:00 P.M. The amount of aluminum hydroxide corresponds to $\frac{1}{2}$–$\frac{3}{4}$ of an ounce, less than the amount recommended clinically in the treatment of gastrointestinal symptoms (5).

* The aureomycin used in this study was supplied by the Lederle Laboratories, Division of the American Cyanamid Company.

The results are presented in Table 1 which includes serum levels and mircogram-hours (mmg.-hours). Microgram-hours were determined by plotting serum levels against time and graphically measuring the area under the curve. Straight lines were drawn between all points and those concentrations listed as less than 0.1 mmg. were plotted as being 0.1 mmg. The test used for measuring serum concentrations will not detect less than this amount.

The results are reported in microgram-hours since this represents the sum of all blood concentrations for each instant of time following a single dose. When comparing two methods of administering a drug, where neither method has a significant effect upon the time-course of the blood concentration and where there is considerable random variation in the time-course, a valid comparison can be made only in terms of concentration-time units. Concentration-time units then give one a single figure representing a set of blood concentration data; a figure which allows a simple statistical comparison of different sets of data.

The data clearly indicate that aluminum hydroxide inhibits absorption of aureomycin from the gastrointestinal tract. The average number of mmg.-hours of aureomycin after an oral dose of 11 mg. per kilogram is 13.7 mmg.-hours. After the same dose of aureomycin plus 0.3 ml. per kilogram of aluminum hydroxide it is less than 3.0 mmg.-hours. The average difference in mmg.-hours produced by taking aluminum hydroxide with the aureomycin is 10.7 mmg.-hours and the standard deviation of this average is 2 mmg.-hours. This difference is significantly greater than zero and the chance of its being zero on the basis of random sampling is less than one in 1400 ($P = 0.0007$).

The most likely explanation of the difference in mmg.-hours produced by giving aluminum hydroxide with the aureomycin is that the aureomycin is bound to the aluminum hydroxide and remains with it in the gastrointestinal tract. It would appear inadvisable to give aluminum hydroxide except for the purpose of eliminating aureomycn from the body.

It is to be noted that women taking aureomycin without aluminum hydroxide had higher serum levels and more mmg.-hours of aureomycin than men after the same per kilogram dose. The average number of mmg.-hours for the women is 19.9 mmg.-hours and for the men 10.4 mmg.-hours. The probability of this occurring on a random basis is

TABLE I

Aureomycin Serum Levels after Oral Administration of 0.3 cc./k. of Aluminum Hydroxide Gel and/or 11 mg./k. of Aureomycin

SUBJECT AND STATUS	TREATMENT	HOURS AFTER TREATMENT					MICROGRAM-HOURS†		
		2	4	8	12	24	A	B	A-B
		*conc. in micrograms/cc.**							
S. D. 79 k. ♂ Fasting	Aureomycin alone	0.8	0.5	0.5	0.5	<.1	9.6		<5.8
	Aureomycin + Al $(OH)_3$	0.5	0.3	0.2	<.1	<.1		<3.8	
J. M. 82 k. ♂ Fasting	Aureomycin alone	1.0	1.2	0.5		0.2	12.6		<9.8
	Aureomycin + Al $(OH)_3$	0.2	0.2	<.1	<.1	<.1		<2.8	
J. S. 77 k. ♂ Fasting	Aureomycin alone	0.5	1.0	0.5	0.5	<.1	10.0		<7.4
	Aureomycin + Al $(OH)_3$	0.2	.1	<.1	<.1	<.1		<2.6	
J. N. 68 k. ♂ Medium Breakfast	Aureomycin alone	0.2	1.0	0.3	0.2	.1	7.0		<4.6
	Aureomycin + Al $(OH)_3$	<.1	<.1	<.1	<.1	<.1		<2.4	
J. C. 67 k. ♂ Heavy Breakfast	Aureomycin alone	1.0	1.0	0.2	<.1	<.1	7.2		<4.8
	Aureomycin + Al $(OH)_3$	.1	<.1	<.1	<.1	<.1		<2.4	
R. L. 69 k. ♂ Heavy Breakfast	Aureomycin alone	<.1	0.5	0.7	1.2	0.2	15.8		<12.2
	Aureomycin + Al $(OH)_3$	<.1	0.5	<.1	<.1	<.1		<3.6	
G. A. 60 k. ♀ Light Breakfast	Aureomycin alone	1.5	3.0	0.8	0.8	<.1	22.0		<19.6
	Aureomycin + Al $(OH)_3$	<.1	<.1	<.1	<.1	<.1		<2.4	
C. W. 53 k. ♀ Light Breakfast	Aureomycin alone	1.5	2.0	0.5	0.5	<.1	15.0		<11.4
	Aureomycin + Al $(OH)_3$	0.3	0.2	<.1	0.2	<.1		<3.6	
M. B. 66 k. ♀ Fasting	Aureomycin alone	2.5	3.0	0.5	1.0	0.2	24.0		<20.8
	Aureomycin + Al $(OH)_3$	0.2	0.2	0.2	<.1	<.1		<3.2	
C. C. 68 k. ♀ Fasting	Aureomycin alone	1.0	2.0	1.0	0.7	0.2	18.6		
Average							13.7	3.0	10.7 ± 2.0‡

* Limit of sensitivity of aureomycin determinations is 0.1 micrograms.

† Column A represents microgram-hours after aureomycin alone; column B represents microgram-hours after aureomycin plus aluminum hydroxide and column A-B represents the difference in microgram-hours produced by taking aluminum hydroxide.

‡ Statistical analysis was done by Mrs. Frieda Eisenberg.

0.003. Differences between males and females are not apparent after taking aluminum hydroxide. The reason for this may be that the concentrations after aluminum hydroxide are at the limit of sensitivity of the method of determining aureomycin. Despite the fact that the number of subjects is small, this difference between males and females is provocative and deserves further investigation.

CONCLUSION

The administration of aluminum hydroxide gel (0.3 ml. per kilogram) with aureomycin (11 mg. per kilogram) reduces the serum mmg.-hours of aureomycin by 80 per cent or more. Following equal doses of aureomycin on a weight basis, females in this series achieved serum mmg.-hours of aureomycin that were twice as high as males.

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MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

In

HURD HALL, THE JOHNS HOPKINS HOSPITAL

MONDAY, MARCH 13, 1950

DR. WILKINS: The first paper of the evening is by Dr. E. A. Bliss, Dr. C. A. Chandler, Dr. M. S. Bryer, Dr. E. B. Schoenbach, and Dr. P. H. Long. The paper will be given by Dr. Long.

THE EXPERIMENTAL BACKGROUND FOR THE CLINICAL USE OF TERRAMYCIN

Terramycin is a new antibiotic which was recently isolated from Streptomyces rimosus and described by Finley and his associates. It will be our purpose tonight to discuss briefly the experimental background for the clinical use of this antibiotic.

The acute toxicity of terramycin in white mice is greater than that of aureomycin or chloromycetin, but less than that of the polymyxins or neomycin. The LD50 of the hydrochloride salt was between 600 and 650 milligrams per kilo when administered by the subcutaneous route. The LD50 of the sodium salt of terramycin, when injected by the subcutaneous route in white mice, was found to lie between 300 and 400 milligrams per kilogram of body weight. Young white rats, which received either 100 milligrams or 200 milligrams per kilogram of body weight of terramycin hydrochloride daily for 14 days, did not develop signs of renal damage.

Bacteriostatic tests *in vitro* show that terramycin has a wide range of antibacterial activity against gram-positive and gram-negative micro-organisms. In general, the antimicrobial effects were similar to those previously noted for aureomycin, with the exception that terramycin was more active against the strains of Ps. aeruginosa which were tested, than was aureomycin. Experiments designed to determine the development of resistance by certain micro-organisms to the antibacterial effects of terramycin *in vitro* showed that at least against the strain of E. coli used in the tests, step-like resistance could be developed in the first culture generation.

In studies designed to test the therapeutic value of terramycin in experimental infections in mice, it was found that this antibiotic was more effective against infections produced by H. influenzae and Kl. pneumoniae than was either aureomycin or chloramphenicol. On the other hand, aureomycin was definitely superior to terramycin as a therapeutic agent for treating experimental infections produced either by Beta hemolytic streptococci or Type I pneumococci in mice.

The administration of a single dose of 2.0 grams of terramycin to each of two

normal individuals produced detectable levels of the antibiotic in their blood for the ensuing 24 hours. During that period, these individuals excreted 11 and 14 per cent respectively of the ingested terramycin.

Terramycin has been given to five individuals in doses ranging from single ones of 2.0 grams to 2.0 grams daily for at least 5 days. All of these individuals have developed nausea, vomiting, and/or looseness of the bowels.

DR. WILKINS: Thank you, Dr. Long. Dr. Long's paper is now open for discussion and questions.

If there is no discussion or question, we will proceed to the next paper, which is by Dr. Helen B. Taussig, Dr. M. A. Engle and Dr. Caroline Bruins. The problem of diagnosis will be presented by Dr. Taussig.

THE CLINICAL SYNDROME PRODUCED BY VALVULAR PULMONIC STENOSIS WITH INTACT VENTRICULAR SEPTUM AND PATENT FORAMEN OVALE

Valvular pulmonic stenosis with an intact ventricular septum and patency of the foramen ovale places great strain on the right side of the heart and causes progressive cardiac enlargement and right-sided heart failure.

The history is significant in that exertional dyspnea precedes the onset of cyanosis; the latter is at first generally transitory, then permanent, and eventually it may become intense. Physical examination shows cyanosis, clubbing, precordial bulge, cardiac enlargement, pulmonic systolic murmur, and pulsations of the liver. There is arterial oxygen unsaturation and polycythemia. Circulation time is prolonged. Electrocardiogram shows high peaked P waves, right axis deviation and right ventricular hypertrophy, or right bundle branch block. Fluoroscopy shows an enlarged right auricle and right ventricle with forceful contractions, a prominent pulmonic conus, and large pulmonary arteries with minimal or absent pulsations.

The significant findings on angiocardiography and cardiac catheterization were presented.

The salient features in the differentiation of this malformation from the tetralogy of Fallot, Ebstein's anomaly of the tricuspid valve, and the Eisenmenger complex were discussed. These patients develop cardiac failure if the Blalock-Taussig operation is performed but may be greatly helped by pulmonic valvulotomy.

DR. WILKINS: Thank you, Dr. Taussig. We will postone the discussion until we have heard the next paper by Dr. Alfred Blalock and Dr. R. F. Kieffer, Jr. on the surgical treatment of the disorder that we have just heard discussed.

SURGICAL TREATMENT OF PULMONIC STENOSIS WITH INTACT VENTRICULAR SEPTUM

The reasons for the difference in the types of treatment that should be used for the tetralogy of Fallot on the one hand and for pulmonic valvular stenosis with intact ventricular septum on the other hand were discussed by the use of lantern slides. This was followed by the showing of a motion picture of a direct attack on the pulmonic valve by the use of the procedure devised by Mr. Russell Brock. The results in sixteen patients in whom this procedure has been used in The Johns Hopkins Hospital were discussed briefly.

(Exhibition of patient and explanation of case by Dr. Kieffer)

DR. WILKINS: These very interesting papers are now open for discussion.

DR. CHESNEY: I have a question. Can you explain to me why the pulmonary artery is dilated beyond the constriction? I don't understand that at all and I would like some light on it if there is an explanation.

DR. BLALOCK: I have said that Dr. Kieffer could answer this better than I and perhaps better than Dr. Taussig, but Dr. Chesney, you should not ask me that question. Dr. Halsted used to hold rounds on this subject and used to speculate as to why an artery dilated distal to a point of obstruction; why, in a patient with a cervical rib, it is the artery beyond it that is dilated. He didn't know the answer and neither do I. But I think if Dr. Halsted had known a lot of physics, and if I knew any, we would be able to give you the answer—at least in part.

It is a jet-like effect. It is the effect of a fine jet of blood coming from a very narrow stenotic area at a tremendous speed and with a tremendous force, but other than that, sir, I am at a loss.

DR. WILKINS: Has anyone else found a good question?

The last paper of the evening is by Dr. W. C. Owens and Dr. Ella U. Owens and will be presented by Dr. W. C. Owens.

RETROLENTAL FIBROPLASIA IN PREMATURE INFANTS

Retrolental fibroplasia, an apparently new disease, has become one of the chief causes of blindness in pre-school children. The disease was first brought to public attention in 1942. Each year approximately 650 premature infants develop the

disease which usually causes blindness in both eyes. It was originally thought that retrolental fibroplasia was present at birth or related to congenital abnormalities. The authors' study made by examining premature infants soon after birth showed that retrolental fibroplasia was not present at birth but developed between the first and fourth month of postnatal life. It was found to be primarily a disease of the retina. The cause of retrolental fibroplasia is unknown but the results of the authors' present investigations indicate that it is the result of an alteration in the metabolism of the premature infant in postnatal life. At present studies are being conducted on the prophylaxis of the disease by the use of d-l alpha tocopherol acetate.

DR. WILKINS: Thank you, Dr. Owens. Are there any discussion or questions on this paper?

DR. LONG: Might I ask a question, although I may appear very dull. Do premature infants who are fed mother's milk get this disease?

DR. WILKINS: Are there any other questions?

DR. OWENS: Retrolental fibroplasia has occurred in infants who have received mother's milk. The amount of tocopherols in mother's milk is very low.

DR. WILKINS: Are there any other questions?

If not, the meeting is adjourned.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

The Neurosis of Man. By TRIGANT BURROW. 428 pp. $7.50. *Harcourt, Brace and Co., New York.*

Dr. Burrow has a reputation in a rather esoteric circle of being a profound thinker with some brilliant concepts that differ markedly from contemporary psychiatric thinking. As the present volume endeavors to sum up thirty years work in "phylobiology" the reviewer was determined to seek the wisdom to be gained from these years of intensive study, even though attempts to read earlier books by the author had been thwarted by the almost insuperable obstacles placed in the way of the reader by the author. The reviewer believes that he has managed to read the book with sufficient care to satisfy himself that it was not worth the effort and would not be, even had it been a brief and lucid essay rather than a cumbersome and redundant 400 pages. The book illustrates the phenomenon that a person who writes with an extremely obtuse but scholarly style can convey the impression of great profundity, particularly when he obfuscates matters by creating an unnecessary and extensive new terminology. In actuality the book and its concepts are extremely naive, filled with sweeping statements as to how attention to phylobiology rather than the misconceptions of psychiatry can lead man to harmonious living and man's world out of its present difficulties.

Stated very simply, the author has found that the trouble with man is that he is a human being. Man's perceptions are biased by culturally transmitted sentiments. Man is selfishly motivated and plagued by the need of maintaining a mask of conformity. He has lost his cohesion to the phylic norm. The evolution of symbolic communication has led to his downfall. In a way, there is no quarrel with these concepts, but it does not seem a promising therapeutic premise to state that the difficulty with human beings is that they are human beings. The author would have people get away from the distorting influence of culture and find some more direct means of communion. The author does not offer any reasonable statement as to how to go about it or just how phylobiology will go about changing things. A rather Yogi-like practice of sitting around in groups with eyes closed and freeing oneself from the phantasies aroused by self-interest may well be a means of relieving tensions, but it does not sound like a very practical way of solving all of the world's difficulties. A simple direct solution such as sought by Dr. Burrow would be very pleasant to attain, but the reader is left with the clear impression that psychiatry and social anthropology will have to continue the laborious task of examining the distortions which interfere with interpersonal relationships.

It seems necessary to remark that the author's comments concerning the objectives and techniques of current schools of psychiatry and sociology seem completely unwarranted. The book shows the result of thirty years of preoccupation with a conviction of illuminating insight, while blocking out objective criticism as being prejudiced, thus preventing realization that the insight is essentially delusional.

T. L.

Physiology of the Nervous System, 3rd edition. By JOHN F. Fulton. 667 pp. $10.00. *Oxford University Press, New York.*

Professor Fulton's textbook has been the standard work in the field since it first appeared eleven years ago. The present edition therefore needs no introduction to those interested in the nervous system. It has been carefully revised throughout and brought up to date. Criticisms of relative emphasis on some subjects and evidence, and sketchy treatment of others, must be expected in any attempt at covering this broad field. These are, however, singularly few in the areas with which the author is personally familiar. The book is well printed. In contains a useful bibliography. It can be recommended strongly.

J. W. M.

The Personality of William Harvey. By GEOFFREY KEYNES. 48 pp. $1.00. *Cambridge University Press, New York.*

This pleasant little volume printed nicely on restful paper, is a splendid small gift (modestly priced at one dollar) and a friendly book to own. Containing black and white prints of eight portraits, and being the Linacre Lecture for 1949, it is a personable sketch of a physician living in the days of witch-burning and firmly resolved to do medical research scientifically. Like the quick images of a flashback, Dr. Keynes gives us some large and small moments in Harvey's life, so that we may compound his personality. Rather engagingly expressed in the conversational yet polite phraseology of a proper don, the little book makes us feel that the velvet gown of Dr. Harvey of St. Bartholomews, physician to Charles I, has brushed us softly for a moment.

B. G. B.

Brucellosis, 2nd edition. By HAROLD J. HARRIS. 617 pp. $10.00. *Paul B. Hoeber, New York.*

The most frequent misinterpretation of laboratory data in infectious diseases by the clinician is brought about by a misstatement of question and answer. The clinician asks the laboratory worker whether a particular patient is or is not suffering from a particular infection. The laboratory data usually merely indicate that that patient has or has not had an antibody response to the suspected organism. In the best situations the laboratory is able to give some information as to when the antibody response took place. Secondly, the laboratory worker may isolate the suspected organism.

Neither of these procedures necessarily relates suspected disease to actual

syndrome at hand. In "Brucellosis" by Dr. Harris, little or no attention is paid to these facts. And yet in no other infectious disease problem has the misinterpretation of question and answer been so great. We are all familiar with the problem of evaluating the neurasthenic individual who lives on a farm where there are demonstrably infected cattle, who has a positive agglutinin titer to Brucella and who gives a history of occasional bouts of fever. We probably have all wondered about the high incidence of positive cultures for Brucella obtained from lymph nodes apparently infected with Hodgkin's disease. What do these facts mean and how should we interpret them?

Dr. Harris has accumulated the story of many patients in this book. They almost all present the same old problem of evaluation. If we are to evaluate the symptomatology, clinical course, diagnosis and treatment of this disease we must do it on the basis of a very rigid exclusion of unproven cases, even though they present varying degrees of suspect symptoms and laboratory findings. Then on the basis of such rigid data we may paint a better picture of the symptomatology of this disease.

It is useless then to discuss the pros and cons of this or that laboratory procedure when the basic problem is unsolved. If you are searching for statements of opinion of many different clinicians who have wrestled with the problem, you will find them here.

F. B. B.

Biological Actions of Sex Hormones, 2nd edition. By HAROLD BURROWS. 615 pp. $8.50. *Cambridge University Press, New York.*

In the last few decades experimental endocrinology has progressed at such a rapid pace that it has become a colossal task to sort out and interpret the more important investigations which lead to a clearer understanding of the subject. Burrows performed a valuable service in 1944 when he wrote "Biological Actions of Sex Hormones" which has been recognized as an authoritative reference work. To bring the subject up to date the *second edition*, written in 1948 but just off the press, required the inclusion of approximately 1000 new references and the addition of 100 pages of text. It is a bit discouraging to realize how much more water has flowed beneath the bridge even since this edition went to press.

Burrows discusses in detail the nature and action of the gonadotropins and the male and female sex hormones but stresses particularly their effects upon the various body structures. The material is well arranged in such a way that it is not difficult for the student to find the information which he seeks. It is probably the most complete reference work in this field of endocrinology. The reviewer's one criticism is that the author, like other biologists, shows a tendency to quote for the most part animal experimentation and to ignore some of the equally important and fundamental information gained by clinical investigation. For example, there is very scant mention of the studies of the metabolic effects of androgen in man or of the knowledge concerning adrenal hormones gained through the investigation of such "experiments of nature" as hyperadrenocorticism.

L. W.

The Practice of Urology. 2 vols. By HARRY C. ROLNICK. 1245 pp. $24.00. *J. B. Lippincott Co., Philadelphia, Pa.*

This new, two-volume encyclopedia of modern urology is the most up to date and complete book of its kind. It seems aimed to replace the classic but now outdated Young and Cabot two-volume works, and the attention to detail shown by the authors together with the pleasing and clear form of its makeup bid fair to justify the hopes of the publishers.

From the early chapters on embryology, anatomy and physiology to the final, 54th chapter on postoperative complications, the material is well organized and thoughtfully written.

The profuse use of a wide variety of well-chosen illustrations enlivens the text and aids the reader in comprehension. The color plates are impressive but might have a broader selection and more general interest. The photographs of pyelograms and other x-rays are generally excellent. For almost each subject the authors have given source references in footnotes fitting the text in a helpful and useful fashion. The index is good and unusually complete.

Every phase of urologic diagnosis is covered. The suggestions as to treatment are usually authoritative and represent the current views. However, treatment is in some instances spottily or incompletely covered. Occasionally controversial issues in treatment are dealt with didactically, but in the main there is a pleasing attention to the *different* ways of doing things. This is particularly well demonstrated in the chapters on the various methods of prostatectomy and the discussion of the factors governing choice of operative approach.

There is a refreshing newness and timeliness about the book. For example, the chapter on "War Injuries" is up to date and well done. There are also excellent chapters on "Female Urology" and "Urology in Children" where the special urological problems of diagnosis and treatment in female and pediatric urology are succinctly discussed. There is a provocative chapter on "Sexual Neuroses," which may make some readers wonder why it is not more complete and why the subject is not more often included in urological texts. Curiously, the author includes a discussion of priapism in this chapter. In the main, however, the material is logically assembled.

The chapter on chemotherapy is good; but as may be expected in these days of many new antibiotics, not completely up to date.

The last half of volume II is chiefly devoted to operative urology. There is an adequate chapter on anesthesia in urology. Chapters 48 to 53 present many operative techniques which are well described and illustrated. However, the operative section is necessarily incomplete and thus emphasizes the need for a good text devoted solely to "operative urology." Flocks' chapter on transurethral surgery is particularly well done and informative.

The two volumes come close to being what the publishers claim for them, an authoritative "source book." The price of the two volumes will put them beyond the reach of most students, and house officers, except as they will find them on

library shelves. However, urologists and the general surgeon or practitioner who desire an authoritative and up-to-date urologic text will find them invaluable.

W. E. G.

Advances in Surgery. Volume 2. Editorial Direction, WILLIAM DEWITT ANDRUS, 590 Pages. $11.00. *Interscience Publishers, Inc., New York.*

In this second volume of *Advances in Surgery* the Editorial Board has maintained its policy of selecting subject matter of timely interest for presentation by leading contributors in the field. Eleven articles are included whose subjects range from *Streptomycin in Surgical Infections* to *Testis Tumors*. Each essay is presented as a monographic review with the addition of extensive data from the authors' personal observations. Riley and Cournand on *Pulmonary Function in Chest Surgery*, Sweet on the *Esophagus*, Smithwick on *Hypertension*, Whipple on *Portal Hypertension*, and Walker and Fisher on *Craniocerebral Injuries* serve to make this an interesting and very informative volume. The book is well illustrated, bibliographies are complete, and there is a good index. It is to be recommended strongly as a reference work in its eleven specific subjects.

H. W. S., JR.

BOOKS RECEIVED FOR REVIEW

Current Therapy. Edited by HOWARD F. CONN. 736 pp. $10.00. *W. B. Saunders Co., Philadelphia, Pa.*

The Cytologic Diagnosis of Cancer. By the Staff of the Vincent Memorial Laboratory of the Vincent Memorial Hospital, Boston, Massachusetts. Published under the Sponsorship of the American Cancer Society. 229 pp. *W. B. Saunders Co., Philadelphia, Pa.*

Endodontia. By BERNHARD GOTTLIEB, SETH LEE BARRON AND J. HOBSON CROOK. 177 pp. $6.00. *The C. V. Mosby Co., St. Louis, Missouri.*

Histology. By ARTHUR WORTH HAM. 756 pp. $10.00. *J. B. Lippincott Co., Philadelphia, Pa.*

A Manual of Cardiology, 2nd edition. By THOMAS J. DRY. 355 pp. $5.00. *W. B. Saunders Co., Philadelphia, Pa.*

Medical Gynecology, 2nd edition. By JAMES C. JANNEY. 454 pp. $6.50. *W. B. Saunders Co., Philadelphia, Pa.*

Medicine of the Year, 2nd issue, 1950. Editorial Direction of JOHN B. YOUMANS. 204 pp. $5.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Occupational Therapy. Principles and Practice. Edited by WILLIAM RUSH DUNTON, JR. AND SIDNEY LICHT. 350 pp. $6.00. *Charles C. Thomas, Springfield, Illinois*

Operative Obstetrics, 5th edition. By J. M. MUNRO KERR AND J. CHASSAR MOIR. 948 pp. $12.00. *The Williams & Wilkins Co., Baltimore, Md.*

Penicillin. Its Practical Application, 2nd edition. By Sir ALEXANDER FLEMING. 491 pp. $7.00. *The C. V. Mosby Co., St. Louis, Missouri.*

The Personality of William Harvey. By GEOFFREY KEYNES. 48 pp. $1.00. *Cambridge University Press, New York.*

Postgraduate Gastroenterology. Edited by HENRY L. BOCKUS. 670 pp. $10.00. *W. B. Saunders Co., Philadelphia, Pa.*

Proceedings of the First Clinical ACTH Conference. Edited by JOHN R. MOTE. 624 pp. $5.50. *The Blakiston Co., Philadelphia, Pa.*

Progress in Biochemistry. By FELIX HAUROWITZ. 405 pp. $7.50. *Interscience Publishers, New York.*

Urological Surgery, 2nd edition. By AUSTIN INGRAM DODSON. 855 pp. $13.50. *The C. V. Mosby Co., St. Louis, Missouri.*

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